

PHYSIOLOGY



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A COURSE
OF
ELEMENTARY
PRACTICAL PHYSIOLOGY
AND HISTOLOGY



A COURSE
OF
ELEMENTARY
PRACTICAL PHYSIOLOGY
AND HISTOLOGY

BY
M. FOSTER AND J. N. LANGLEY.

SEVENTH EDITION

EDITED BY
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PREFACE TO THE SEVENTH EDITION.

IN this Edition, most of the Lessons have been re-written, and a few have been added. But the general arrangement, planned by Professor—now Sir Michael—Foster in the first edition has been kept. One divergence from the original plan has been made, namely, the omission of the Lesson on the Dissection of the Rabbit and Dog. The specialization of study which has taken place in the last twenty-five years seemed to make this omission inevitable; but the conclusion was reached with regret.

Some of the directions are given in small type, they refer on the whole to the less elementary work. But each Teacher will decide for himself, according to the stress of his teaching and the resources of his laboratory, which sections best come in a first year and which best come in a second year course.

The portions of the book dealing with chemical physiology, and with the physiology of muscle and

nerve have been in large part revised or written by Dr Shore, whose name in consequence has been added to the Title-page. The proofs of the sections on chemical physiology have been kindly read by Dr F. Gowland Hopkins, to whom we owe many valuable suggestions. A similar kindness has been rendered us by Dr W. F. R. Rivers in the Lessons on the physiology of the special senses.

In the histological part of the book I have had invaluable assistance from Miss Greenwood—now Mrs Bidder—who has given me the benefit of her experience of the difficulties which beset the elementary student, and who has corrected the proofs with a care which I cannot too gratefully acknowledge.

I desire also to thank Mr W. B. Hardy and Dr H. K. Anderson for suggestions on various points.

J. N. LANGLEY.

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LESSON I.

STRUCTURE OF CORPUSCLES OF BLOOD AND OF LYMPH.

A. CORPUSCLES OF FROG OR NEWT.

Read the Notes on the Use of the Microscope in the Appendix, p. 354.

1. A frog is given you the brain and spinal cord of which have been destroyed (cp. App. p. 376). Take the frog in one hand, and with a stout pair of scissors cut off a foot at the ankle joint. Touch a clean slide with the cut surface, so as to leave a *small* drop of blood on the slide. Place on it a cover-slip.

Later, drops of fresh blood should be obtained by cutting off the other foot, the leg at the knee, the fore arm at the elbow-joint; lastly, blood should be obtained direct from the heart; in order to do this cut through the skin in the median ventral line, cut transversely through the lower part of the sternum just above the epigastric vein, with stout scissors cut through the sternum in the middle line and so expose the heart; cut off the tip of the ventricle, and touch a slide with

it. For §§ 5, 6, 8, blood which has flowed from one of the cut surfaces will serve.

Examine the mounted drop of blood under the microscope with a low magnifying power¹ and observe the numerous corpuscles floating in the plasma.

Examine it with a high magnifying power¹ and observe the **red corpuscles**; if a large drop of blood has been taken the corpuscles will overlap one another, in which case another preparation should be made with a smaller drop.

a. The red corpuscles are flattened ellipsoids; note their spindle shape when seen on edge as they roll over. The great majority are of the same size and tint.

b. They appear at first homogeneous, but soon a certain number show a central paler, oval nucleus.

c. A single corpuscle is pale yellow, the colouring substance (hæmoglobin) being equally diffused throughout it; when several corpuscles lie over one another they together appear red.

2. Examine the **colourless corpuscles** in parts of the specimen where the red are not very numerous.

a. They are much fewer than the red. They are smaller than the red, but they vary in size.

b. They are usually spherical, when first mounted, but soon many of them put out processes and become irregular in form.

¹ For convenience the term 'low power' will be used throughout for a combination of lenses which magnifies 50 to 100 diameters, and the term 'high power' for a combination of lenses which magnifies 250 to 500 diameters.

c. The majority have no distinct granules (**hyaline corpuscles**). Some have comparatively large and refractive granules; these are the **coarsely granular corpuscles**.

d. The nucleus can seldom be made out, though it may be seen if the corpuscle is very extended. Do not confound a heap of granules or a protuberance with the nucleus.

e. Choosing a corpuscle, either elongated or having several processes, watch carefully its amœboid movements; make half-a-dozen drawings of its outline at intervals of about twenty seconds. As a rule the coarsely granular corpuscles put out few and rounded processes, and the finely granular corpuscles put out more numerous and more pointed processes.

f. If it is desired to watch the movements for an hour or so the drop should be protected from evaporation. (a) Melt a little glycerine jelly (cp. App. p. 372) on a warm bath, keep the cover-slip in place by gently holding a lifter against one edge, and with a small brush, brush a little glycerine jelly over the edges all round. (β) If the slide is to be warmed, use melted paraffin (of low melting point) instead of glycerine jelly. Before applying the paraffin, dry the slide at the edges of the cover-slip with blotting-paper, and warm the slide. Olive oil may be used instead of glycerine jelly or paraffin.

3. *Hanging drop.* Cut out a number of pieces of blotting-paper about 3 cm. by 2 cm. Place them together so as to make a pad. With scissors or cork borer cut out from the centre of the pad a hole a little smaller than the cover-slip to be used. Dip the pad in salt solution 0.6 p.c. and place it on the slide. On the centre of a cover-slip place a small drop of lymph or blood, and lower the cover-slip over the hole in the pad, so that its edges rest on the pad, and the drop of lymph hangs in the centre and does not run off to the pad. Thus a small moist chamber is

formed. The pad should be kept wet by adding a little water to one edge from time to time. Examine the movements of the white corpuscles under the microscope.

4. Lymph crowded with white corpuscles, many in active movement, may be obtained in the following way. The brain of a frog is destroyed, and the wound plugged with cotton-wool. A drop of curari is injected under the skin; this paralyses the lymph hearts, so that the lymph accumulates in the lymph sacs. The frog is kept in a dish containing water about $\frac{1}{4}$ inch deep, for half-a-day to a day. Lymph may be obtained from any of the lymph sacs, but, if the frog has been placed in a normal position, in especial quantity from the ventral sacs, beneath the skin of the abdomen. Since the lymph clots quickly when withdrawn, not more should be taken up in the pipette than is required at the time.

The white corpuscles which have ingested foreign particles will be seen, if a drop of milk or if a drop of water containing hay bacillus, or Indian ink, be injected into a lymph sac about an hour before examining the lymph. The early stages of action may be seen by mixing a little lymph and bacillus culture, and at once examining in a hanging drop.

5. **Irrigation.** Mount another small drop of blood. Place a small drop of .1 p.c. acetic acid on the glass slide so that it just touches the edge of the cover-slip; place a piece of blotting-paper on the opposite side just touching the fluid at the edge of the cover-slip, the acetic acid will then run under the cover-slip and mix with the blood. Note the changes which take place.

a. In the **colourless corpuscle**, the cell substance becomes more transparent but shows some irregular granules; a granular nucleus comes into view. As a rule the nucleus is horse-shoe shaped in the coarsely granular cells; and is round or consists of clumps connected by thin threads in the hyaline cells.

b. In the **red corpuscles** the nucleus becomes obvious; it is when first seen nearly homogeneous, and oval in outline, later it becomes granular and usually irregularly rod-shaped.

c. The red corpuscles swell up owing to absorption of water, most after a time become spherical (if strong acid be used the corpuscles usually preserve their shape).

d. They become colourless, the hæmoglobin being dissolved from the stroma; occasionally the hæmoglobin is massed round the nucleus before complete solution takes place (effect of water), and occasionally it stains the nucleus yellow (effect of acetic acid).

e. Finally the outline of the corpuscles is seen as a faint line at some distance from the nucleus. Observe the not infrequent excentric position of the nucleus.

f. Some corpuscles are much more readily acted on than others.

6. Irrigate a drop of blood, first with 30 or 50 p.c. alcohol and then with a strong aqueous or alcoholic solution of Spiller's purple or magenta.

The red corpuscle becomes spherical and its peripheral rim, the *pseudo-membrane*, stains. The nuclei both of the red and of the colourless corpuscles stain deeply.

7. Place several very small drops of blood two or three mm. apart on a slide and leave for a few minutes, then cover with a cover-slip, and put under a high power. Take a little blood from a freshly killed frog and establish a current underneath the cover-slip from

one side of it to the other (cp. § 5). The first small drops will have partially clotted and will serve as an imperfect barrier to the corpuscles in the current; in such places note that the shape of the red corpuscles is easily changed and recovered, and that the colourless corpuscles stick to one another and to the glass more than do the red. After the current has passed for a short time largish clumps of colourless corpuscles will be seen.

8. Evaporate to dryness on a slide a drop of a strong solution of urea; on this place a small drop of blood, mount quickly, and observe the breaking up of the red corpuscles into spheres; sometimes a corpuscle will put out a varicose filament which breaks up later into spheres.

9. '*Æcoid*' and '*zoid*.' Let a little fresh blood of a frog or newt run into about five times its volume of a 2 p.c. aqueous solution of boracic acid, and mount *at once* a drop of the mixture. The corpuscles, nearly normal in appearance when first mounted, rapidly become altered; in all or nearly all cases the hæmoglobin leaves the stroma or the outer part of it, and becomes accumulated around the nucleus. As this is taking place the hæmoglobin may form a star-shaped figure in the stroma; from some corpuscles, but as a rule, from a few only, the mass formed by the nucleus with the hæmoglobin, *i.e.* the so-called '*zoid*,' is extruded from the colourless '*æcoid*.'

In mammalian corpuscles a 2 p.c. solution of tannic acid causes a similar separation of the hæmoglobin from the stroma.

10. Dilute a little fresh blood with twice its volume of .6 p.c. salt solution; mount a drop of the mixture and place it aside for an hour or so to clot; irrigate it with 30 p.c. alcohol and then with Spiller's purple dissolved in water or in dilute alcohol. Note the deeply stained network of fibrin fibrils and the numerous long threads of fibrin running from the broken-down colourless corpuscles.

11. Irrigate a drop of blood with 95 p.c. alcohol; a granular precipitate will be formed in the stroma. Irrigate with Spiller's purple, the precipitate will stain deeply.

12. **Preparation of a dry film of blood.** Make a film preparation in one of the following ways.

a. Moisten with blood a small glass rod (1 to 2 mm. in diameter) and rub it over the surface of a clean cover-glass. The film of blood should be so thin that it dries in a few seconds on waving the cover-slip in the air.

b. Take two clean cover-slips. Hold one in forceps, and touch a small fresh drop of blood with the centre of the glass; place it on the other cover-slip and at once withdraw it laterally.

13. **Staining a film with eosin.** See that 3 small wide-mouthed bottles are ready, the first containing a saturated solution of eosin in 75 p.c. alcohol, the other two containing 75 p.c. alcohol. Have ready also a pad of half-a-dozen pieces of smooth filter or blotting-paper, without fluff. Take up the cover-slip with forceps and hold it for a minute in the eosin solution, let the excess of fluid drain off (5 seconds), hold the cover-slip for 5 seconds in the first bottle of alcohol and for 5 seconds in the second bottle of alcohol. Then place without delay between the pads of filter-paper and lightly press to remove excess of fluid. Wave the cover-slip in the air to dry it. When dry place a small drop of Canada balsam on the centre of the cover-slip, and lower this slowly on a glass slide.

Note the deep red stain of the granules of the coarsely granular leucocytes (**the oxyphil granules**).

The red corpuscles should be obvious, and there should not be any precipitate.

14. *Staining a film with eosin and methylene blue.* Add to the bottles used in § 13, two, one containing methylene blue¹ in 75 p.c. alcohol, the other 75 p.c. alcohol. Proceed as in § 13, but after the cover-slip has been placed in the second alcohol hold it for 10 seconds in the methylene blue, dip it for one second in the 75 p.c. alcohol, then without delay press lightly between the blotting pads. The nuclei of the various cells will be stained blue. If cells with basophil granules are present, the granules will also be stained blue (cp. p. 58, § 10).

15. *Preservation of hæmoglobin in red corpuscles by heat.*

Place a dry film of blood for 5 to 10 min. at a temperature of 120° C. This may be obtained by placing a Bunsen flame under one end of a long strip of copper; the part of the strip which is at a temperature of 100° C. can be determined by noting where a small drop of water boils; the specimen should be placed a little nearer the flame than this, the filmed side uppermost. (Instead of this, the cover-slip is sometimes passed twice through the flame of a Bunsen burner.) The film may be stained as in § 13 or § 14.

The fixation of the hæmoglobin in the corpuscles may be observed later in sections of tissues hardened in osmic acid vapour, osmic acid, mercuric chloride dissolved in salt solution, Müller's fluid, formol.

16. **Platelets.** Mount a small drop of blood direct from the blood vessels or from the heart (cp. § 1). Examine at once. Note the elongated pale cells (platelets) about $\frac{1}{3}$ the size of the red corpuscles. Some are found singly, others sticking together in groups. The nucleus can generally be made out, it is large in comparison with the amount of cell substance. The nucleus soon becomes round and the cell substance indistinct.

¹ A saturated solution of methylene blue in 75 p.c. alcohol, diluted with an equal bulk of 75 p.c. alcohol.

17. Let a drop of fresh blood of a frog or newt drop into 5 c.c. of a mixture containing 0.6 p.c. NaCl, 0.6 p.c. peptone, 0.02 p.c. methyl-violet; and stir at once. In this fluid the platelets are preserved for a day or so. The nuclei of all the elements of the blood are stained rather deeply; the cell substance of the white corpuscles and of the platelets takes a light stain (cp. 3, § 5).

18. The platelets and other corpuscles of the blood may also be preserved by allowing a drop of blood to run into 5 c.c. of (1) a mixture containing 0.5 p.c. osmic acid, and 0.75 p.c. NaCl., (2) 1 p.c. oxalate of potassium. In the former fluid they do not stick together, and to a slight extent only in the latter.

Permanent preparations may be made from the blood mixed with osmic acid. The mixture is allowed to stand for a day; the fluid decanted, water is added to the residue and shaken; when the corpuscles have settled, the water is poured off; the process is repeated; 50 p.c. alcohol is then added, in this the corpuscles can be kept till required; with a little care the corpuscles can be stained.

B. CORPUSCLES OF MAN.

1. With a sharp needle¹ prick the finger a few millimetres from the root of the nail; touch with the centre of a cover-slip the drop of blood which issues; lower the cover-slip on a slide (cp. A. § 1). Observe the **red corpuscles**.

a. They roll about readily when the cover-slip is lightly touched. Soon after being taken from the body they stick to one another, and, owing to their shape, usually in rouleaux.

¹ It is better to use the triangular needle specially made for the purpose.

b. They are biconcave discs. Note that on focusing down on the circular face a darkish centre and a light rim is first seen and then a light centre with a darkish rim: when viewed in profile and the centre focussed they appear somewhat dumb-bell shaped.

c. They appear homogeneous, their colour is like that of the red blood corpuscles of the frog (cp. A. § 1, *c*).

d. Towards the outside of the drop, where evaporation is going on, many of the red corpuscles are crenate.

e. They are much smaller than the red corpuscles of the frog.

2. Observe the **colourless corpuscles**. They are larger than the red, they resemble in general appearance the white corpuscles of the frog (A. § 2, *c. d. e.*); to observe their amœboid movements a drop should be protected from evaporation (A. § 2, *f*) and, preferably, warmed to the temperature of the body.

In stained specimens there will be seen small cells with a spherical nucleus (lymphocytes), a few hyaline cells, a considerable number of finely granular oxyphil cells, and a few coarsely granular cells.

3. Irrigate with .5 p.c. acetic acid (cp. A. § 5).

a. The red corpuscles swell up and become spherical, their hæmoglobin is dissolved, leaving the hardly visible stroma. (Effect of water.)

b. No nucleus is brought into view.

c. In the white corpuscles the cell-substance becomes more transparent, and the nucleus comes into view.

4. *Platelets.* Kill a rat or mouse with chloroform. Expose the heart, and cut through the pericardium. Cut through the ventricle and let one drop of blood fall into 5 c.c. of (*a*) methyl-violet solution (A § 17), (*b*) osmic acid and salt (§ 18), (*c*) 1 p.c. oxalate of potassium. Stir and examine a drop for platelets. They are small, colourless, oval bodies, $\frac{1}{4}$ to $\frac{1}{3}$ the size of the red corpuscles and without nuclei.

5. Wash the tip of the finger, place on it a drop of the methyl-violet solution or of the potassium oxalate solution. Prick the finger through the drop. As soon as blood comes transfer to a slide and examine.

DEMONSTRATIONS.

1. Circulation of blood in the mesentery of the newt to show the platelets sticking for a variable time to the walls of the vessels (cp. Lesson XVIII. § 9, *b*).

2. Specimens to show amphibian and mammalian platelets.

3. Specimens to show the chief stages of indirect nuclear division in tissue cells of larval salamander (Flemming's fluid¹).

4. Hanging drop to show leucocytes with ingested bacilli.

5. Stage for varying temperature of hanging drop, and passing gases over it.

¹ The fixing agent in which the tissue is placed on being removed from the body is, here and later, given in brackets. The details of treatment are given in the Appendix.

LESSON II.

MEASUREMENT OF OBJECTS UNDER THE MICROSCOPE. COUNTING BLOOD CORPUSCLES. SOME CHARACTERS OF BLOOD.

1. **Drawing and Measurement of Objects under the Microscope.** Place a drawing-board (made so that the drawing surface is at an angle of 15° to 20° with the table), in front of the microscope, and touching its foot, or at an ascertained distance from it. Fix a piece of smooth paper in the middle of the drawing-board.

A simple form of camera lucida is shown in Fig. 1. Slip the ring (*r*) over the tube of the microscope (*t*) then replace the eye-piece (*o*). Arrange the brass rod carrying the prism (*A*) on the left-hand side and shift the prism till (1) the edge (*a*) stretches half-way over the lens of the eye-piece, (2) the lower angle of the prism nearly touches the edge of the eye-piece, (3) the anterior exposed surface of the prism is parallel with the drawing-paper. Then turn the prism round the vertical axis so as to uncover the eye-piece.

Focus with a low power a stage micrometer, *i.e.* a

millimetre divided on glass, into tenths, and one of the tenths divided into hundredths. When the lines are

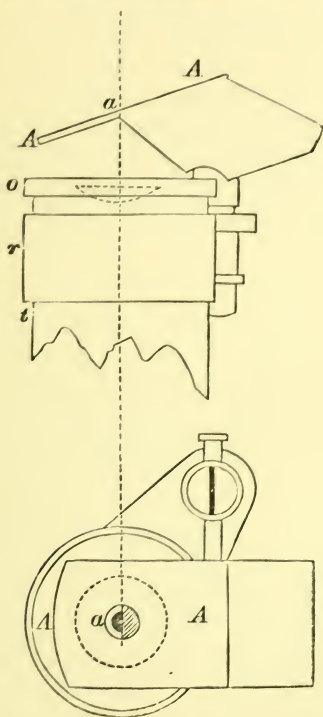


Fig. 1. The upper sketch shows the camera from the side, the lower sketch shows it from above.

seen distinctly, turn the prism back again into position. On looking down the microscope with the eye close to *a*, the lines of the stage micrometer and the point of a pencil placed on the drawing-paper should be visible.

If they are not, watch the lines carefully, for the appearance of the pencil point over them as the prism is turned slowly and slightly around the horizontal axis, the point of the pencil at the same time being moved; try also the effect of shading the drawing-paper.

Draw the $\frac{1}{10}$ th micrometer lines. Then place the drawing alongside a millimetre scale, and note the length in millimetres of the scale drawn. If it measures 80 mm., then the scale drawn, which on the micrometer was one millimetre, has been magnified 80 times.

2. Draw similarly on another piece of paper the lines of the stage micrometer with the high eye-piece, and objective. It will be sufficient to draw the lines indicating $\frac{1}{100}$ th of a mm. Ascertain the magnification of the drawing. If the $\frac{1}{100}$ th of a mm. drawn measures 38 mm., the magnification will obviously be 380.

3. Substitute now a fresh preparation of human blood for the stage micrometer, and draw some red corpuscles under the high eye-piece and object-glass; taking care that the drawing-board and paper are in the same positions as in § 2. Fold the scale drawn in § 2 at right angles to, and through the lines; and measure with it the diameter of the corpuscles you have drawn. One division of your scale is $\frac{1}{100}$ mm. *i.e.* $10\ \mu$. (μ = micron, $\frac{1}{1000}$ mm.) The red corpuscle drawn should occupy rather less than one division of your scale since the red corpuscle is ordinarily 7 to $8\ \mu$ in diameter.

4. Write on each scale drawn, the eye-piece and object-glass with which it was drawn, and the position of the drawing-board.

Later, in drawing microscopic objects, draw them in the condition of one of the two scales. Thus the scale will allow the diameters of the object to be at once measured.

The phrase *magnifying power of the microscope* is used for the magnification of the scale, when it is drawn on a level with the stage of the microscope.

5. *Measurement of size of objects by means of an ocular micrometer.* The ocular micrometer consists of a disc of glass with a scale engraved on it. A ledge to receive it is placed in the eye-piece on a level with the focus of the upper lens.

Take the high eye-piece and objective, place the ocular micrometer face downwards in the ocular. Note the number of divisions of the ocular and of the stage micrometer which correspond exactly with one another. Reckon from this how many of the smaller divisions of the stage micrometer,—each of which is 10μ —one division of the ocular micrometer corresponds to. Thus if 5 ocular divisions cover exactly 4 stage divisions; 1 ocular division = $\frac{4}{5} \times 10\mu = 8\mu$, and a human red blood corpuscle would almost exactly be covered by one division of the ocular micrometer. If the microscope has a draw tube, draw it out to its full length, and redetermine the value of the ocular divisions. Determine similarly the value of one division of the ocular micrometer with the low ocular and objective.

6. **Reaction of blood.** Prick the finger and touch the drop of blood with litmus paper made of glazed paper and neutral litmus (see App. 374). After 10—20 seconds remove the drop of blood with a cloth or with distilled water. The paper is not stained with hæmoglobin and a blue patch is seen showing that the blood is alkaline.

7. *Determination of the alkalinity of blood.* A decinormal solution of tartaric acid (0.75%) is prepared; 1 c.c. of this should be exactly neutralised by 0.004 gr. NaHO. Dilute this tenfold with

water. Take a series of watch-glasses and into each put 1 c.c. of the $\frac{1}{100}$ normal acid as follows:—

1. 0.9 c.c. $\frac{1}{100}$ normal tartaric acid + 0.1 c.c. Na_2SO_4 sol.
2. 0.8 c.c. " " " + 0.2 c.c. " "
3. 0.7 c.c. " " " + 0.3 c.c. " "

and so on. Add to each watch-glass 0.1 c.c. of freshly drawn blood. After stirring determine by litmus paper which is neutralised by the blood. The amount of acid which neutralises 0.1 c.c. of the blood is thus found, and since the amount of NaHO which will neutralise this acid is known, the alkalinity of 100 c.c. of blood can be expressed as equivalent to a definite amount of sodium hydrate.

The alkalinity of normal human blood is equivalent to .2 to .3 gr. per cent. of NaHO .

8. *Determination of the specific gravity of blood.* A series of solutions of glycerine and water of specific gravity varying between 1035 and 1068 are prepared. Pour a little of one of them, of sp. gr. 1050 for example, into a narrow but deep glass vessel. Prick the finger and suck up a drop of blood into a fine glass pipette, the point of which is bent at right angles to the main tube. Gently blow the drop of blood out of the pipette so that it issues horizontally into the middle of the glycerine solution. If the blood immediately sinks, take a glycerine solution of higher sp. gr., if it rises, one of lower sp. gr. and make another observation with another drop of blood. After a few trials a solution will be found in which the blood neither sinks nor rises immediately. The blood is of the same sp. gr., as this solution.

9. *Enumeration of the red corpuscles.* Count the number of red corpuscles with Thoma's hæmacytometer in the following manner.

See that the counting cell and the pipette are clean and have ready a large coverslip and a watch-glass containing the diluting fluid. This may be sodium sulphate solution of sp. gr. 1025, sodium chloride .8% solution, or Hayem's fluid, which consists of 1 part of sodic chloride, 5 parts of sodic sulphate, .5 parts of corrosive sublimate in 200 parts of water.

Prick the finger and when a sufficiently large drop of blood

is formed, gently suck blood into the pipette as far as the mark 1. With a cloth or the finger remove any blood adhering to the point of the pipette. Wash the blood on into the diluting chamber of the pipette with the diluting fluid selected, gently turning the pipette to assist the mixing and very slowly draw the fluid up exactly to the mark 101. Put the finger on the tip of the pipette, leaving the capillary tube full of the diluting fluid, and by gently shaking thoroughly mix the blood and the fluid, taking care that the contents of the capillary tube are not drawn up into the diluting chamber. The blood is thereby diluted 1 in 100. Allow the contents of the capillary tube and a few drops of the diluted blood to escape, and then at once carefully place a small drop of blood on the centre of the counting chamber. Put on the cover-slip by a sharp lateral thrust; this assures its close apposition to the wall of the chamber and Newton's colour rings should appear. Allow two or three minutes for the corpuscles to settle, then count the number of corpuscles in at least 10 adjacent squares, including in each square any corpuscles overlapping the upper and the left boundary lines but, by way of compensation, excluding those overlapping the lower and right boundary lines.

In order to facilitate the process of counting the squares are divided into groups by lines bisecting every fifth horizontal and vertical column of squares.

Determine the average number found for a square. Since each square has an area of $\frac{1}{400}$ sq. mm., and the depth of the cell is $\frac{1}{10}$ mm., the cubic contents of the fluid on each square is $\frac{1}{4000}$ cub. mm. Hence the average number of corpuscles found multiplied by 4000 gives the number in 1 cub. mm., of the diluted blood, and 100 times this is the number in 1 c.mm. of the blood.

10. *Enumeration of the white corpuscles.* Dilute the blood 1 in 10 with the mixing pipette provided for the purpose, using the same precautions as before, and taking care that a large drop of blood is collected before the point of the pipette is put into it. The same diluting fluids may be used, but the counting is rendered more easy if .3% acetic acid is used since this renders

the red corpuscles invisible. Count the number of white corpuscles lying on the large squares formed by the lines bisecting every fifth horizontal and vertical column of small squares. Count the number on ten of these large squares, using a rather low power unless the microscope is fitted with a mechanical stage. Calculate the average number for a large square. A large square has an area of $\frac{1}{16}$ sq. mm., and a cubic content of $\frac{1}{160}$ cub. mm. Hence the average number found multiplied by 160 gives the number in 1 cub. mm., of the diluted blood, and 10 times this is the number in 1 c.mm. of the blood.

11. If Gower's hæmacytometer is used proceed in the following manner.

Fill the larger pipette with sodium sulphate solution of sp. gr. 1.025 up to the mark on the stem, it then contains 995 c.mm.; empty it into the measuring glass. Fill the small pipette with freshly drawn blood up to the line marked 5 c.mm.; empty it into the measuring glass, and with the fluid in the measuring glass wash out the blood sticking to the inside of the tube; thoroughly mix the blood and salt solution with the glass spatula, place a small drop of the mixture in the centre of the glass cell and over it lay a cover-slip, arrange the springs on the cover-slip to keep it in position, and under a high power count the number of red corpuscles in ten of the squares which are marked at the bottom of the glass cell.

Since the depth of the cell is $\frac{1}{2}$ mm. and the side of each square is $\frac{1}{16}$ mm., there is beneath each square $\frac{1}{160}$ c.mm. of the mixture, *i.e.* $\frac{1}{16000}$ c.mm. of blood, hence the number of corpuscles in 10 squares multiplied by 10,000 gives the number of corpuscles in 1 c.mm. blood.

12. *Determination of the relative amount of hæmoglobin.* Determine the relative amount of hæmoglobin in the blood with Gower's hæmoglobinometer in the following manner. Put a few drops of water into the graduated tube. Prick the finger and let a large drop of blood accumulate. By means of the pipette suck up 20 c.mm. of blood and eject it into the graduated tube. Draw up distilled water into the pipette and wash it out two or three times into the graduated tube. Thoroughly mix the blood and the water in the graduated tube and then add water drop by drop, mixing after each addition until the tint in the graduated

tube is the same as that of the tube of standard tinted jelly. The reading of the level of the fluid in the graduated tube gives the relative amount of hæmoglobin in the sample of blood in percentage of the normal amount.

DEMONSTRATIONS.

1. Determination of the specific gravity of the blood (see § 8).
2. Enumeration of the corpuscles (see § 9).

LESSON III.

SERUM. CHARACTERISTICS OF PROTEIDS. CLOTTING OF BLOOD.

1. Observe the clotting of freshly shed blood¹, it is at first fluid but soon passes into a jelly which gradually becomes firm; if then placed aside for some time, drops of clear serum will, by the shrinking of the fibrin, be pressed out on the surface of the clot; later the clot shrinks more or less completely from the vessel, squeezing out more and more serum.

2. With a pipette remove from the clot a quantity of serum. It is colourless in the rabbit, yellow in the dog and horse, reddish yellow in the ox, and yellow in man. Frequently it is coloured by the discharge of hæmoglobin from the red corpuscles.

3. Determine the specific gravity. This varies somewhat, but is about 1028 in man, 1030 in the ox.

4. Dilute serum tenfold with water², and with it observe the following general **reactions of proteids**.

¹ This will be obtained by the Demonstrator.

² The addition of this amount of water usually makes the fluid slightly cloudy from the precipitation of a small amount of paraglobulin.

a. Xanthoproteic reaction. Take a little of the dilute serum, add a few drops of nitric acid, and boil. The white precipitate of proteid material at first formed becomes yellow and partially dissolves, forming a yellow solution. If the quantity of proteids present is small, the yellow solution only will be obtained. Place the test-tube in a stream of water from a tap to cool, and when cold add ammonia; the yellow is turned to orange.

b. To another small quantity of the serum add a few drops of Millon's re-agent¹. A precipitate will be formed which turns pinkish on boiling; if the amount of the proteids present be small, no distinct precipitate will be formed but the fluid will turn pink on boiling.

c. Add a drop of cupric sulphate solution to an excess of sodium hydrate. To the blue solution so formed add a little dilute serum, the fluid will become violet (cp. albumose and peptone, Lesson XXII). The violet colour becomes deeper on warming.

d. Add strong spirit, a precipitate is formed (peptone in solution is precipitated with difficulty).

e. Add excess of acetic acid and a few drops of a strong solution of potassium ferrocyanide, a precipitate is formed (peptone is not thus precipitated).

5. *a.* Place a test-tube containing diluted serum in a beaker of water and heat the water not too slowly to about 80° C. By means of a thermometer placed in the test-tube observe the temperature at which the

¹ See Appendix, p. 374.

solution becomes opalescent, but note that the precipitate does not become particulate nor the fluid clear.

b. Just acidulate diluted serum with a drop or two of weak acetic acid and repeat the experiment. The coagulation now becomes complete, the precipitate running together leaving a clear fluid. Note that the coagulation chiefly takes place at 73° C. to 75° C. Coagulation however begins at 70° C. and is not complete until 82° C.

6. *a.* Take some serum in a beaker and saturate it with magnesium sulphate, stirring to assist the solution of the salt. **Paraglobulin** is precipitated. When the saturation is complete filter, or dilute with an equal volume of saturated MgSO_4 solution and filter. Wash the paraglobulin with saturated MgSO_4 solution on the filter.

b. Dissolve the paraglobulin by adding a little water and so forming, with the salt adhering to the precipitate, a dilute MgSO_4 solution.

c. Determine the coagulating point of the solution as in § 5. *b.* Paraglobulin coagulates at 75° C.

d. Dilute serum tenfold with water, and taking a rather large quantity cautiously add a drop or two of acetic acid. At a certain point of acidification a precipitate of paraglobulin occurs. It dissolves if too much acid is added. Let the fluid stand, decant, and filter off the precipitate. Take the precipitate up in distilled water, it does not dissolve. Add a particle of salt (NaCl or MgSO_4), the paraglobulin passes into solution.

e. Let a drop of serum fall into a large quantity of distilled water. A faint cloud due to precipitated paraglobulin is seen.

7. Take the filtrate from § 6. *a.*, dilute it with water and heat it. A coagulation of **serum albumin** takes place.

8. Dilute the filtrate from § 6. *a.* with five or six volumes of water and determine its coagulating point. Serum albumin in a fluid containing the same percentage of salts as serum coagulates at 73° C. but the presence of a large amount of MgSO_4 in this fluid lowers the coagulating point. Large dilution with water brings the coagulating point nearer to the normal, but this will not be actually reached unless the salt is largely removed by dialysis.

9. Half saturate serum with ammonium sulphate by adding to it an equal volume of a saturated solution of the salt. Paraglobulin is precipitated. Filter. Add solid ammonium sulphate to the filtrate to saturation. Serum-albumin is precipitated. Filter; apply the xanthoproteic and other proteid tests to the filtrate. No proteids are now present.

10. *a.* **Clotting of blood.** With a feather stir *slowly* about 10 c.c. of freshly shed blood¹; a considerable portion of the blood will form a clot on the feather; squeeze out the clot under a stream of water from a tap; the clot shrinks considerably and a small quantity only of **fibrin** is obtained.

b. Repeat the experiment, but this time stir *quickly*, filaments of fibrin will be obtained; note that the fibrin

¹ This will be obtained by the Demonstrator.

is extensible and elastic; leave the defibrinated blood for a day, no further clot is produced.

11. Apply the xanthoproteic and Millon's test for proteids (cp. § 4) to fibrin chopped up and suspended in water.

12. Take two test-tubes and in each place a few flocks of fibrin.

a. Add water and place in a water-bath at about 30° C. for a day; the fibrin does not dissolve (it thus differs from albumin and peptone).

b. Treat similarly but with dilute (1 p.c.) solution of sodic chloride; the fibrin does not dissolve (it thus differs from globulin).

13. Place two or three flocks of fibrin in a test-tube containing a few c.c. of .2 p.c. HCl, the fibrin soon swells up and becomes transparent; neutralize the acid with Na_2CO_3 , the fibrin shrinks to its original size. If the fibrin is warmed with the acid, solution *slowly* takes place, acid-albumin being formed (cp. Less. x. p. 81).

14. Examine the plasma of horse's blood kept, by means of cold, from coagulating¹.

¹ The blood is allowed to run from the animal into a tall narrow vessel contained in a much larger one packed with ice, a little salt may be mixed with the ice, but of course not enough to reduce the temperature so much that the blood is frozen; sometimes also a vessel filled with ice is placed in the one which receives the blood. Horse's blood is preferable to bullock's or dog's, since it clots less readily and the red corpuscles sink more quickly. Clotting sometimes takes place, but the remaining fluid may still give a clot on appropriate treatment.

a. Transfer with a pipette 2 or 3 c.c. of the plasma into a small test-tube. Observe the coagulation as the temperature rises. Avoid shaking. Probably the fibrin will adhere so strongly to the sides of the tube that little contraction will take place. On being freed from the glass it will contract. If the clot has already shrunk away from the sides of the vessel, it may since it is colourless be overlooked unless the fluid be carefully examined.

b. Dilute 1 c.c. of the plasma with 50 c.c. of distilled water or normal saline solution. Carefully avoid shaking and leave it till the next day. Observe the fine delicate fibrils of fibrin which are formed.

15. Examine the plasma of blood prevented from coagulating by the presence of neutral salts¹.

a. Remove 1 or 2 c.c. carefully with a pipette, avoiding blood-corpuscles as much as possible, and dilute five to tenfold with water. The mixture will clot, probably in about half-an-hour, if placed in the warm chamber; more slowly if left at the ordinary temperature.

b. Determine the temperature at which plasma coagulates as in § 5. *b.* A coagulation of *fibrinogen* occurs at 56° C. Filter off the coagulated fibrinogen. Dilute the filtrate as in § 15. *a.* It does not clot however long it is left.

c. Half saturate plasma with NaCl by adding an equal volume of a saturated solution of the salt. Fibrinogen is precipitated. Let the tube stand a few minutes, then filter. Dissolve the precipitate in the dilute saline solution formed by adding to it a very little water.

¹ In preventing coagulation by neutral salts, blood is collected in a vessel containing a saturated solution of magnesian sulphate; as the blood runs in, it must be mixed well with the salt solution, preferably by stopping the flow of blood now and then and turning the vessel upside down. There should be about 1 vol. of the salt solution to 4 vols. of blood. If sodic sulphate is used an equal volume of the saturated salt is mixed with the blood. The corpuscles are separated from the blood by centrifugalisation and the plasma pipetted off.

d. Set some of the fibrinogen solution prepared in this way in the warm bath. After a short time clotting may take place.

16. Examine the plasma of blood prevented from clotting by the precipitation of the calcium salts¹.

a. Dilute a little decalcified plasma with two or three vols. of water and add a few drops of 1% calcium chloride solution and place the tube in the warm bath. The plasma clots in a few minutes.

b. Prepare fibrinogen from decalcified plasma by half saturation with NaCl as in § 15. *c.* Dissolve the precipitated fibrinogen by adding a very little water and divide the solution into two parts. To one part add a drop of calcium chloride solution and set the two tubes in the warm bath. The part to which calcium chloride has been added clots, the other does not.

17. *a.* Take some fibrin washed free from entangled hæmoglobin and let it soak in a 5% solution of NaCl in the warm bath for some hours. Strain away the fluid. This contains *fibrin ferment*.

b. Throw some serum into alcohol and let the precipitated proteids stand in the alcohol for some days. Decant off the alcohol and dry the precipitate on a filter-paper in the air. Extract the precipitate with a little water, filter the aqueous extract. It contains fibrin ferment.

c. Add a few drops of a fluid containing fibrin ferment to diluted salted plasma and set the tube in the warm bath. Clotting takes place much more quickly than without added fibrin ferment. Salted plasma when diluted usually clots slowly because it contains a little, but only a little, fibrin ferment.

¹ The blood is received into a vessel containing a 1% solution of potassium oxalate, 10 c.c. of the solution to every 100 c.c. of blood to be collected, and mixed by gentle shaking. The plasma is obtained by centrifugalisation.

d. In a similar way show that added fibrin ferment hastens the clotting of fibrinogen solutions. Fibrinogen solutions prepared as in § 15. *c.* may clot because some fibrin ferment may be already present.

e. Heat the solution containing fibrin ferment to 65° C. The ferment is destroyed and the solution no longer hastens the clotting of diluted salted plasma.

18. *a.* Add to hydrocele fluid or to other exudation fluids which have not clotted spontaneously some solution containing fibrin ferment and put it in the warm bath. In a few minutes the fluid clots¹.

b. Prepare fibrinogen from hydrocele or other exudation fluid as in § 15. *c.* and add to its solution fibrin ferment and put it in the warm bath. In a short time the fibrinogen clots. The clotting of isolated fibrinogen may be most readily obtained by extracting it from exudation fluids.

DEMONSTRATIONS.

1. The action of albumoses, when injected into the vascular system, of preventing the clotting of blood.

2. Intravascular clotting caused by the injection of extracts of lymphatic glands and nucleo-proteids.

¹ Some samples of hydrocele fluid do not clot if fibrin ferment only is added.

LESSON IV.

STAINING AND MOUNTING SECTIONS.

1. Staining sections and mounting them in Canada balsam. Sections are given you in 75 p.c. alcohol¹. Take seven watch-glasses and fill them respectively $\frac{1}{3}$ full with (1) Delafield's **hæmatoxylin** diluted with four volumes of diluting fluid², (2) **picrocarmine**³, (3) water, (4) 30 p.c. alcohol, (5) 50 p.c. alcohol, (6) 75 p.c. alcohol, (7) 95 p.c. alcohol. Pour a few drops of clove oil into another watch-glass. Cover up the 95 p.c. alcohol with a watch-glass to prevent evaporation.

Lift up four sections on a glass rod 1 to 2 mm. in diameter³ and place them in 50 p.c. alcohol for a minute; transfer them in the same way to 30 p.c. alcohol for a minute; then transfer two sections to hæmatoxylin, and

¹ Sections of spleen hardened in potassium bichromate may be taken for §§ 1 to 8, and the results compared.

² For the method of preparing staining fluids see Appendix, p. 365.

³ Instead of a glass rod, a glass tube finely drawn out may be used, the end being closed by fusing in a Bunsen flame.

two, first to water, and afterwards to picro-carmin. The sections in hæmatoxylin will be stained in about a quarter of an hour, those in picro-carmin in half-an-hour to an hour¹.

With the fine glass rod, take a section from the hæmatoxylin and place it in 30 p.c. alcohol for a minute, gently moving it. Place it for the same time in 50 p.c. and in 75 p.c. alcohol. Leave it for 3 to 5 minutes in 95 p.c. alcohol. Take it up on the glass rod, gently touch the end of the watch-glass with it to remove excess of alcohol, and place it in clove oil,—the *clearing agent*—for 2 or 3 minutes. In the clove oil the folds in the section will usually disappear.

If the section is folded when in 50 p.c. alcohol, it should be transferred from fluid to fluid on a lifter instead of on a glass rod. Arrange the section flat on a lifter, hold it in place by touching it lightly with a needle as the lifter is raised from the fluid; let excess of fluid drain from the lifter (taking care that the section does not become dry); and then lower the lifter gently into the next fluid.

Take a section from picro-carmin and move it gently in water, before placing it in 30 p.c. alcohol; after this treat it in the same way as the section from hæmatoxylin.

¹ Delafield's hæmatoxylin undiluted stains sections in 1 to 2 minutes, Ehrlich's acid hæmatoxylin, and Mayer's carmalum in $\frac{1}{2}$ to 1 hour. The stain with the acid hæmatoxylin will be deeper, if the sections are washed with tap-water instead of distilled water. The rate of staining varies with the agent used to fix the tissue. Tissues fixed with alcohol or mercuric chloride stain more quickly than those fixed in a fluid containing chromic acid or osmic acid.

With a lifter transfer the sections from the clove oil to a slide, and examine them under a low power of the microscope. They should be transparent throughout. If either has any opaque spots, put it on the warm bath for a few minutes. If the opaque spots—which are caused by the presence of water—do not disappear, the section should be thrown away¹, and another passed from the staining agent through the alcohols, especial care being taken to remove all excess of 95 p.c. alcohol before it is placed in the clove oil.

The complete dehydration of the sections is rendered more certain by placing them in absolute alcohol after 95 p.c. but absolute alcohol is expensive and is not necessary, since clove oil will take up a small quantity of water. When xylol instead of clove oil is used as the clearing agent, greater care must be taken to dehydrate the specimen.

When the sections are transparent, tilt the slide slowly and let the clove oil run off, keeping the sections in place with the aid of a needle. Let the slide stand vertically on a piece of blotting-paper for a minute or two to drain: with the clean-cut edge of a piece of blotting-paper remove the clove oil around the specimen. Clean a cover-slip by rubbing it on a smooth, hard surface, with a piece of clean silk or linen. Let a small drop of fluid Canada balsam dissolved in xylol fall on the section. With the aid of a needle gently lower the cover-slip on the balsam.

¹ The section may be dehydrated by removing it from the clove oil to 95 p.c. alcohol (or better, absolute alcohol), for about 10 minutes and then replacing it in clove oil; but the student is recommended to take a fresh section.

2. Place a section for half-an-hour in each of the following fluids, acid hæmatoxylin, alum carmine, carmalum. Mount them (cp. § 1) and compare the staining.

3. *Staining with methylene blue and with saffranin.* With these—and a number of other reagents—it is generally best to overstain the sections and then to decolorize them to the required extent. Sections are left in a solution of saffranin¹ in 50 p.c. alcohol or in 1 p.c. aqueous solution of methylene blue for 2 to 24 hours. They are then passed through the series of alcohols. In these the colour is more or less rapidly extracted and the sections must be passed through the fluids the more quickly the more rapidly the extraction is observed to take place. Clove oil dissolves these staining agents, so that cedar wood oil or xylol, in which they are insoluble, should be used as the clearing agent, in place of clove oil. Sections of a young salamander may be stained; the changes in the nucleus during division will be seen.

4. **Staining sections and mounting them in glycerine.** Take two sections stained as in § 1, remove them from the staining agent to water, stir the water with a glass rod till no more colouring matter comes from the section (a minute or two); then (a) place one in a watch-glass with a drop of glycerine, and move it gently about till the glycerine has penetrated it; place a *small* drop of glycerine on a slide, to this remove the section on a needle, spread out the section, and cover with a cover-glass. Sections stained with any carmine stain may advantageously be mounted in formic glycerine (glycerine containing 1 p.c. of formic acid 1·16 sp. gr.).

b. Treat the other section similarly, but with

¹ A saturated solution of saffranin in 50 p.c. alcohol, diluted with an equal volume of 50 p.c. alcohol.

dilute glycerine (equal volumes of glycerine and water) instead of with strong glycerine; use a lifter to transfer the section to the slide; remove excess of fluid and cover.

5. When it is desired to keep sections mounted in glycerine, they may be treated in the following way. Place the slide and a small bottle of glycerine-jelly on a warm bath. There should be no glycerine beyond the edges of the cover-slip, but if there is, remove it with blotting-paper. With a small brush, brush a little glycerine-jelly round the edges of the cover-slip. Put the slide aside for a day or more, then brush gold size (zinc white, Brunswick black, Canada balsam will also serve) over the glycerine-jelly.

6. **Double staining with hæmatoxylin and eosin.** Arrange watch-glasses as in § 1. Add one containing a saturated solution of eosin in 50 p.c. alcohol. Stain rather deeply with hæmatoxylin; after the stained section has been placed in 50 p.c. alcohol, place it in the eosin solution for one minute, then back again in 50 p.c. alcohol. Pass through the stronger alcohols, the clove oil and mount. If the section when in 95 p.c. alcohol has a blue tinge only, it should be placed once more in the eosin and passed more quickly through the alcohols. The nuclei are stained with hæmatoxylin, the cell substance and most other tissue with eosin.

If the tissue has been hardened in Müller's fluid, potassium bichromate, or by brief treatment with osmic acid, the hæmoglobin of the red corpuscles—which in these cases is usually preserved—will be stained orange with eosin.

7. *After-staining with picric acid.* Picric acid may be used after any other stain. It is perhaps best after carmine or

hæmatoxylin¹. The section is stained and treated in the usual way up to 75 p.c. alcohol. From this it is removed to a watch-glass containing picric acid² dissolved in 95 p.c. alcohol. After staying about two minutes in this, it is placed in 95 p.c. alcohol and moved about till a faint yellow tinge only is left. It is then transferred to clove oil and mounted.

Picric acid stains red corpuscles brilliantly when the hæmoglobin is preserved, it stains also elastic tissue and the horny layer of the skin; it stains muscular tissue more readily than white fibrous tissue; and striated duct cells more readily than the alveolar cells of glands. In slight excess it stains the whole section.

8. *Hæmatoxylin and rubin S³ with picric acid.* Stain with hæmatoxylin. After the section has been passed through alcohols up to 75 p.c., place it for one minute in the rubin mixture. Transfer it to 95 p.c. alcohol, and treat in the usual way. If required, a little more picric tint may be given by treating it as in § 7, after it has been stained with the rubin mixture. Rubin stains especially the connective tissue, and is best used when a small amount only of this tissue is present.

9. **Mounting separately, stained sections imbedded in paraffin⁴.** *a.* Place a section in a watch-glass, and pour over it a few drops of turpentine. See that the balsam and a clean cover-slip are ready. With a lifter transfer the section to a slide, tilt the slide and wipe away the excess of turpentine, then add a drop of balsam, and cover with a cover-slip.

¹ A piece of arytenoid cartilage may be taken.

² A 2 p.c. solution of picric acid in 95 p.c. alcohol.

³ Also called acid-magenta, and acid-fuchsin. For proportion of mixture see Appendix, p. 369.

⁴ A compact or fairly compact structure should be taken, as a piece of liver, pancreas, ureter or bladder, hardened in potassium bichromate. The piece should be stained with hæmalum for a day before imbedding, for § 9, and left unstained for § 10.

b. Mount another section, using xylol instead of turpentine. Xylol¹ is very volatile, and the section after removal of excess of xylol will rapidly dry on the slide and become useless unless this is prevented by the addition of balsam. Xylol is preferable to turpentine for tissues that have been treated with osmic acid, since turpentine is apt to dissolve some of the stained fatty substances.

10. *Staining separately sections imbedded in paraffin.* Take two or three sections and dissolve the paraffin with turpentine as in § 9. Take up the sections on a thin glass rod, touch blotting-paper with them to remove excess of turpentine and place in 95 p.c. (or absolute) alcohol in a watch-glass. Leave for 5 to 10 minutes, then pass through successively weaker alcohols, stain and mount as in § 1 (cp. App. p. 371, for another method).

11. Mounting serial stained sections. Place a *small* drop of collodion dissolved in clove oil, on a slide. With a small brush (or with the finger) rub the drop so that it forms a very thin film over a portion of the slide a little larger than a cover-slip. Take the ribbon of nine sections² given you, and cut it with a scalpel into three series of three sections each. Place these on the film of collodion so that the second series of three is below the first, and the third below the second. Press a small brush lightly on the centre of each section and roll it to right and left, so as to flatten the sections and make them stick to the slide. Place the slide on a warm bath at a temperature just sufficient to melt the paraffin. When the paraffin is melted, dip the slide into a bottle

¹ Benzol, toluol, and a mixture of four parts of turpentine with one part of creosote, are also used to dissolve paraffin.

² A piece of small intestine hardened in mercuric chloride may be taken; stained with hæmatoxylin for §§ 11, 12; unstained for § 13.

containing turpentine, for one to two minutes. Then wipe the turpentine from the lower surface of the slide, and stand the slide up to drain. Wipe away the turpentine on either side of the sections. Place a line of dilute balsam on a cover-slip near one edge; let this edge first touch the slide, and gently lower the cover-slip so that the balsam flows over the sections.

12. Flattening folded sections. *a.* Clean the slide by pouring on it a drop or two of strong spirit, and rubbing with a clean cloth. Place a flat basin of distilled water over a small flame, regulating the flame so that the temperature of the water is 35° to 37° C. Place the sections on the water, they will spread out and become quite flat. Dip the slide in the water obliquely, draw the sections to the slide with a needle, hold the upper edge of the paraffin on the slide with the needle and gently lift the slide out of water, with the sections on it. Stand up the slide to let the water run off, so that the sections are in contact with the glass, place between smooth pads of blotting-paper, and touch lightly to remove most of the remaining water and to make the tissue adhere to the glass. Place on a bath at about 35° C. for half-an-hour or longer to dry. Then place on a warmer bath to melt the paraffin and mount as in § 11.

b. Clean a slide; pour water on it, place the sections in the water. Place the slide on a warm bath. As the water becomes warm the sections will flatten. As soon as the sections are flat, remove the slide, let the water run off, and treat as after the corresponding stage in *a*. With delicate sections it is better not to remove excess of water with blotting-paper, but after

draining, to let the sections dry in the warm bath ; in this case they should be left at 35° C. till next day. The drying period may be shortened thus : after the slide has been at 35° C. for half-an-hour (i) dip it in 95 p.c. alcohol, drain, and put on the bath for another half-an-hour or (ii) dip in absolute alcohol, drain, put in turpentine at once.

c. Proceed as in *b*, but use 50 p.c. spirit instead of water.

13. *Staining serial sections on the slide*¹. *a*. The several fluids required (turpentine or xylol, alcohols, staining agents) are arranged in a row in bottles without necks, and of such size that the slide just fits into them.

Proceed at first as in § 12, either *a*, *b*, or *c*. If the slide taken was clean and the process has been properly carried out, the sections will in most cases adhere to the slide during the subsequent staining and mounting.

After the paraffin has been dissolved in turpentine or xylol ; wipe off excess of fluid and place in absolute alcohol for two or three minutes ; transfer for a minute to 95 p.c. alcohol, and so on through the successive alcohols to the staining agent, *e.g.* Delafield's hæmatoxylin. When the section is stained wipe away excess of staining fluid, pass back through the alcohols, removing excess of fluids at each transfer. After absolute alcohol, leave the slide in turpentine or xylol for a couple of minutes ; then stand it up to drain ; mount as in § 11.

Some sections, especially those of tissues which have been kept long in osmic acid or in chromium compounds, are apt to come away from the slide when treated as above. In such cases a fixative is used as in *b*, and *c*, following.

b. If the sections do not require to be flattened, rub the slide with a mixture of egg-albumin and glycerine in the manner in which collodion was used in § 11.

¹ The sections may be fixed to the cover-slip instead of to the slide. This method does not require so large a quantity of the various fluids, and there is less chance of their being spoilt.

c. If the sections require flattening, proceed as in § 12, *b*, using dilute egg-albumin (2 c.c. filtered white of egg in 100 c.c. water) instead of water.

In either case when the paraffin has been dissolved from the sections, leave the slide for 5 to 10 minutes in absolute alcohol, then pass through alcohols and stain as in *a*.

In this process the alcohol fixes the sections to the slide by coagulating the film of egg-albumin. The drawback of the process is that the egg-albumin is stained by most reagents.

LESSON V.

SECTION CUTTING AND IMBEDDING.

1. Preparation of Hardened Tissue for Freezing. If the piece of tissue to be cut has been kept in alcohol, place it in 50 p.c. and then in 30 p.c. alcohol, each for about 10 minutes. Remove the tissue to water for 1 to 3 hours in order to extract the alcohol. The extraction is hastened by placing on the warm bath, at about 38° C. Place then in dilute gum¹ for an hour to a day as convenient.

Successful section cutting depends largely upon using a sharp razor, it should not be markedly hollow ground, or it may bend and give sections consisting of bands of unequal thickness. The razor should be placed flat on a microscope stage, and the edge examined with

¹ The gum solution is prepared by dissolving gum arabic in sufficient warm water to make a freely flowing solution, and filtering through linen. When it is required to keep the piece of tissue in a given position whilst freezing, it may be transferred to a thick solution of gum a few minutes before placing it in the plate of the microtome; it can then be kept in position by a needle for the short time that elapses before it is frozen. Since gum alone freezes to a hard mass, a little sugar solution may with advantage be added to it.

a low power, if there are notches in it it requires re-grinding.

2. Ice and Salt Freezing Microtome. Ice is broken into fragments about the size of walnuts; salt is sprinkled liberally over the ice; and the mixture packed in the box of the microtome¹. The plate of the microtome remains below freezing-point for 2 to 3 hours.

Clean the plate of the microtome and the grooves in it. Place the piece of tissue (given you in gum) in the centre of the plate. If there is much gum around the tissue cut it away after it is frozen.

Take the tripod frame which carries the razor, and see that the razor is firmly fixed by the screws. Then adjust the level of the razor by means of the three screws supporting the frame in the following manner:—

Screw up the front screw till $\frac{1}{2}$ to $\frac{2}{3}$ of it is below the frame. Adjust the back right-hand screw so that the right-hand edge of the razor will just cut into the frozen gum over the tissue; adjust similarly the back left-hand screw; repeat these two adjustments, so that whatever part of the razor is used in cutting, sections of nearly the same thickness will be obtained.

Hold the frame firmly, but without unnecessary pressure, and push it straight forward over the tissue, this should cut off a portion of the gum. Lift up the frame—or its anterior end,—carry it back, turn downward the front screw $\frac{1}{8}$ th of a revolution, push the

¹ The freezing mixture made by pounding together equal parts of ice and salt causes too great a lowering of temperature, and the frozen tissue is less easy to cut.

frame straight forward again, and so on. In a short time, sections of the tissue will be obtained. With a small brush moistened with water remove each section, as it is obtained, from the razor, and float it off in a dish of water. A number of sections may be cut, and these removed together from the razor, but the sections are apt to become entangled and they are generally less perfect than the sections which are removed separately as they are cut.

After cutting some sections, of a thickness corresponding to $\frac{1}{6}$ th of a revolution of the front screw, cut others, turning the screw less than $\frac{1}{6}$ th between each section and note the smallest angle through which the screw can be turned, complete sections still being obtained.

Stain and mount the sections. (See Lesson IV.) It must be remembered that the gum unless dissolved out of the sections will form a granular precipitate in them when they are treated with strong alcohol.

After the sections are cut, carefully clean the razor and the plate of the microtome.

The proper rate to carry the razor through the tissue varies with the temperature of the frozen mass; when it is a few degrees only below zero, the movement may be the quickest possible; when it is frozen hard, it should be carried slowly through the tissue, otherwise the sections are apt to curl or break up.

Note. In cutting large sections, it is best to place on the razor a number of small drops of water and to cut slowly; the section folds up on the razor. The razor (with the frame) should then be dipped under water and the section floated off; it should be taken out on a glass slide and treated on the slide with 30, 50, 75 p.c. alcohol, etc.;

care should be taken to remove as much as possible of the clearing agent, otherwise the Canada balsam in which the section is mounted may remain a long time fluid.

3. *Ether Freezing Microtome.* The fine nozzle of the ether spray apparatus is easily stopped up by fine particles of dust. To avoid this, see that the bottle to contain the ether is clean, and filter into it the ether to be used.

The piece of tissue should not be more than 3 to 4 mm. thick ; for the upper surface of pieces thicker than this freezes slowly.

Before beginning the ether spray, adjust the razor (see § 2) so that the edge just touches the top of the tissue as it lies on the plate ; place a brush and dish of water in readiness. Then set the ether spray going by means of the hand bellows, keeping the second bag of the bellows just pressing against the net surrounding it. The tissue should be frozen in about a minute, the time varying with the temperature of the room. If the freezing does not begin rapidly look at the spray, if there is not a good jet, the nozzle of the apparatus probably requires cleaning out.

As soon as the tissue is frozen, begin cutting sections as with the ice and salt microtome. Immediately the gum on the plate at the edge of the tissue begins to lose its dense white colour, cease cutting sections, and ply the ether spray to re-freeze. Then cut more sections.

When the sections are cut, clean the razor and the microtome plate.

4. *Cutting Fresh Tissues.* A piece of tissue fresh from the body may be placed direct on the microtome plate and frozen. It is best to cut it a few degrees only below freezing-point. The ice rapidly blunts the razor, and ice crystals are apt to distort and tear delicate structures. This can be more or less avoided by placing the piece of tissue for 5 to 15 minutes in dilute gum, this however causes some changes in the tissue cells.

a. Cut sections of *e.g.* a piece of rat's kidney. Transfer the sections to salt solution 0.9 p.c. and with a brush gently unfold them. Dip a slide obliquely under a section, with a needle hold one corner of the section on the slide, lift up the slide, if necessary pull out the edges of the section so that it lies flat and unfolded,

drain off the salt solution. Treat another section in the same way.

Place a cover-glass on one of the sections and examine it. (A drop of dilute methylene blue or Spiller's purple may be added to another section, in 5 minutes the drop drained away, and a cover-glass put on.) Let two or three drops of 95 p.c. alcohol fall on the other. Leave it for a quarter of an hour or longer, then stain *e.g.* with hæmatoxylin and mount in Canada balsam.

b. Cut sections of *e.g.* a small lymphatic gland. Transfer the sections to 30 p.c. alcohol. With a brush move them about in the alcohol to remove some of the leucocytes. Stain and mount. (Sections after unfolding in 30 p.c. alcohol may be transferred flat on a lifter to any of the usual hardening agents.)

5. Imbedding in Paraffin. Take the piece of tissue given you, which has already been stained in bulk¹. Place it for $\frac{1}{4}$ hour in 95 p.c. alcohol, and for $\frac{1}{4}$ hour in absolute alcohol. Transfer it to a little turpentine² in a watch-glass, cover up and place on a warm bath at about 45° C. for 5 to 10 min. Place on the warm bath a pair of forceps and a piece of blotting-paper. Take up the tissue with the warm forceps, drop it on the blotting-paper, and roll it over, wipe the points of the forceps, take up the tissue and drop it into one of the dishes of melted paraffin in the paraffin bath. The paraffin used should have a melting point of about 45° C. Leave the tissue in the paraffin for $\frac{1}{2}$ hour to 3 hours according to its size.

¹ The piece of tissue should if practicable only be a few millimetres thick. It is stained in bulk by leaving it for 1 to 2 days in acid hæmatoxylin or alum carmine. It is washed in a stream of water for several hours, and then passed through the alcohols (cp. p. 29).

² When the student has become accustomed to imbedding, xylol or cedar-wood oil should be used in place of turpentine (cf. Less. iv. § 9, b).

Rub the inside of a watch-glass with glycerine, removing excess so that the glycerine only remains as a smear. If in the paraffin dish there is more than about half a watch-glass full of paraffin, pour the excess into another dish. Light a Bunsen burner, and have ready a piece of wire or an old scalpel. Pour the paraffin with the tissue into the watch-glass; warm the wire in the Bunsen flame, and with it adjust the tissue so that the face to be cut is towards the edge of the watch-glass; and if air-bubbles are present remove them by means of the heated wire. If the paraffin begins to set round the tissue, when an air-bubble still remains attached to it, heat the wire and melt all the paraffin around the tissue.

Carefully take up the watch-glass and place it in a shallow dish of cold water; when the paraffin has set, immerse the watch-glass cautiously in water; then place the glass surface under a stream of water from a tap. The more quickly the paraffin is cooled, the less chance there is of crystals forming, and the easier it will be to cut.

Cut away the paraffin on each side of the tissue, and remove the block with the imbedded tissue. Cut the paraffin into a rectangular block, cutting away the paraffin close up to the tissue on the face to be cut and on the adjoining faces, but leaving 2 to 3 mm. of paraffin on the face opposite to that to be cut.

A piece of smooth glass is given you with a central mass of paraffin fixed to it. Heat a scalpel in a Bunsen burner and melt the central part of the paraffin, on this place the paraffin end of the block with the imbedded tissue. Pass the heated scalpel once or twice

along the point of junction of the paraffin mass and the block, to melt it thoroughly and make a good union. With the heated scalpel, heap up a little paraffin about the base of the block. Leave for a minute, then cool under the tap.

6. Cutting sections. Take the tripod razor, adjust as in § 2, and cut sections in a similar manner, carrying the razor *rapidly* through the tissue, and using the same portion of the razor. The successive sections will stick together and form a ribbon. When a ribbon of half-a-dozen sections has been made, place it aside on a piece of paper, and cut a fresh ribbon. The sections are mounted as in Lesson IV. § 9, 11, or 12.

Note. The method given above, viz., of pouring paraffin with the tissue into a watch-glass serves very well for small objects ; but as the surface of the watch-glass is curved, it is better when objects more than half a centimetre in length are to be imbedded, to use instead of the watch-glass, either a small card-board or paper box, or two L-shaped imbedding frames. The latter are made of brass or of lead, with sides about a centimetre high ; they are placed on a glass slide so that the long limb of each is in contact with the short limb of the other, they thus enclose a space, the size of which can be varied according to the size of the tissue ; into the space paraffin with the tissue are poured, and the tissue is adjusted by a hot scalpel.

7. The Rocking Microtome. Note the mechanism of the microtome (Fig. 2). Turn up and down the large screw at the end of the lever, and note the extent of the movement of the anterior end of the lever which carries the paraffin. Turn similarly the milled wheel at the base of the screw. Pull the handle backwards and forwards, note that in pulling it forward, a catch engages with the milled wheel and pushes it round, *i.e.* pushes the paraffin on the lever towards the razor. Move

backwards and forwards the guard of the catch, the position of this determines how far the wheel is pushed round by the catch, *i.e.* the thickness of the section. Pull the handle forward, and then slowly move it back, counting the clicks of the catch as it passes over the teeth of the milling; adjust the guard so that there are 8 to 10 clicks. Eight teeth give sections approximately 5μ in thickness.

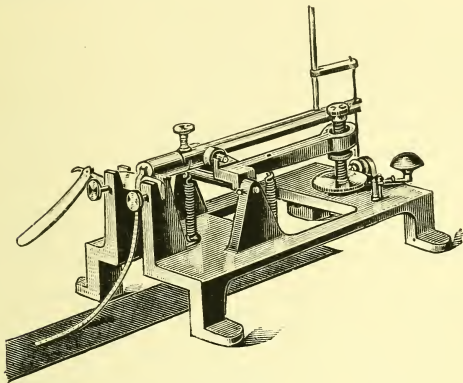


Fig. 2.

Procedure. Imbed the tissue as in § 5, and shape the paraffin into a rectangular block. Take the paraffin carrier from the microtome. On this fix the imbedded tissue, thoroughly melting the adjoining surfaces of the paraffin by means of a heated knife. With a sharp scalpel, carefully shape the part of the block surrounding the tissue so that the surface to be cut is rectangular.

Screw the large screw of the microtome downwards as far as it will go. Fix the carrier to the microtome, adjusting it so that the paraffin reaches nearly up to the position to be taken by the razor. Fix the razor firmly in the frame. Leave the right arm on the end of the lever carrying the paraffin so as to bring the paraffin on a level with the edge of a razor. Adjust the paraffin carrier so that the edge of one block to be cut is parallel to the edge of the razor and about half a millimetre from it; in doing

this, do not put the left hand over the razor, but adjust the carrier from the right-hand side. Screw up firmly the carrier. Push the guard of the catch back as far as it will go, and move the handle until the first section is obtained, then adjust the guard so that the catch turns the milled wheel 8 to 10 teeth. Each forward movement of the handle now gives a section which is flat and adheres to the preceding section. When 3 or 4 are obtained, lift them up with a forceps, so that the ribbon of sections runs free of the razor.

The chief causes of bad sections are (1) over-hardness of the tissue; this was normal to the tissue, or produced by warming in agents such as xylol, or to overheating in the warm bath; (2) imperfect imbedding; this may be due to a lack of sufficient penetration of the paraffin, to crystals of paraffin having been formed, or to the presence of small bubbles of air. The surface of the block to be cut should be examined with a lens after it has been shaped, if patches of crystals or bubbles are visible it is best to re-imbed; (3) to bluntness or notches in the razor.

8. *Cutting sections in paraffin melting at 58° C.* Paraffin of high melting point allows thinner sections to be cut than paraffin of low melting point. On this account paraffin melting at 58° C. is sometimes used. But sections of the paraffin melting at 58° C. are apt to curl and do not adhere to a ribbon at ordinary room temperatures. They form a ribbon, however, if the block is coated with paraffin of a low melting point.

Imbed a piece of tissue in paraffin melting at 58° C., shape the block, and fix it in the usual way to the carrier of the microtome. Then dip the block up to the base in melted paraffin of melting point about 40° C., leave it there for 2 to 3 seconds, then lift it out, and hold it vertically for about 10 seconds for the paraffin to set. Put it aside for a minute or two to cool (or cool it in water, removing afterwards the adhering water). With a sharp scalpel cut away the soft paraffin from the surface to be cut, and from the sides of the block, leaving it only on the sides which will be parallel to the razor edge in cutting. Examine the junction of the hard and soft paraffin with a lens. If the two do not adhere, the process of re-coating should be repeated.

9. *Superficial Imbedding.* This can be employed with small firm pieces of tissue. It is often advantageous with portions of the nervous system treated by the Golgi-Cajal method (cp. p. 341). The piece of tissue is placed in 95 p.c. (or absolute) alcohol for 5 to 15 minutes. The imbedding mixture consists of 2 parts of hard paraffin and 1 part of vaseline. Sections of this curl less than sections of hard paraffin; and fold less than soft paraffin.

Take a short oblong or cylindrical block of paraffin-mixture. Scoop out a small hole at an end of it. Take the tissue up with forceps, roll it over on blotting-paper to remove excess of alcohol, but without allowing it to dry, dry the points of the forceps, and place the tissue in the hole in the paraffin. With a warm suction pipette, fill the hole with melted paraffin mixture. Heat a needle or wire in a Bunsen flame; and with it remove any bubbles that may be present, being careful not to touch the tissue with the wire when it is overheated. Let the paraffin cool a little, then cool it further in water. Pare down the paraffin to the surface of the tissue, and level the edges. Blow strong alcohol on the razor from a wash-bottle and cut sections free hand, removing the section to 95 p.c. alcohol; the tissue must throughout be kept moistened with alcohol. The sections can be at once transferred to clove oil and mounted, or if the paraffin has penetrated into the outer layers, they may be transferred to absolute alcohol, then to xylol and mounted. If an unstained piece of tissue has been taken the sections may be stained in the usual way.

LESSON VI.

HYALINE CARTILAGE.

1. Snip off a piece of the free edge of any of the thin cartilages projecting from the sternum or shoulder-girdle of a freshly killed young newt¹. Gently scrape away with a scalpel any tissue attached to it.

a. Mount it in .6 p.c. sodium chloride solution² and with a high power note that

The **matrix** is studded at tolerably regular intervals with **cartilage cells** or **corpuscles**.

Each corpuscle consists of a spherical or ovoid mass of **cell substance**, in which lies a relatively large **nucleus**. Both of these are fairly clear and transparent. Most of the cells entirely fill up the cavities in which they lie. Along the cut edge some cavities from which the cells have fallen out may generally be seen. Except at the free edge, the cells are two or more layers deep.

¹ Instead of this, sections of the head of the humerus or femur of any young animal may be made.

² See note below.

The matrix is hyaline or faintly granular, and is comparatively (cp. §§ 3, 4) in small quantity.

If the newts are not quite young the cartilage will vary from the above in the quantity of matrix, the shape and arrangement of the cells, and in the cells possessing small fat globules and often two nuclei.

b. Irrigate the piece with acetic acid 1 p.c. The following changes will be seen as the irrigation proceeds:

The nucleus becomes much more granular and distinct.

The cell substance becomes granular and more or less hides the nucleus.

The cell substance becomes transparent.

The cell substance shrinks from the matrix, and presents an irregularly serrated border. Note the space thus formed between the cell and the matrix.

Note. **Examination of fresh tissues.** When a piece of tissue is examined fresh, in order to see as nearly as possible its appearance during life, it must on no account be washed or mounted in water, since water causes rapid and extensive alteration.

The best method, as a rule, is to remove a small piece of fresh tissue as quickly as possible to a slide, and to put on a cover-slip at once without adding any fluid. Drying may be retarded by putting a little moist blotting-paper round the edges of the cover-slip.

When fluid is added to the tissue, it should be one which causes as little change as possible. A .6 p.c. to .75 p.c. solution of sodium chloride in tap water is used for the tissues of the frog and newt, and a .75 p.c. to .9 p.c.

solution for the tissues of the mammal. These are called **normal salt solutions**. The tissue should not, if it is avoidable, be soaked in the normal salt solution, but only moistened with it.

In most cases, fresh aqueous humour, and fresh blood serum, may with advantage be used, instead of normal saline.

2. Place a small piece of newt's cartilage in gold chloride solution .5 p.c. for about half-an-hour (until it is of a light yellow colour), then wash well with water, and place it in a vessel containing water just acidulated with acetic acid; leave it exposed to the light. When it has become a red-purple colour, mount it in formic glycerine. The reduction takes a few hours only in bright sunlight, and one to two days in cloudy weather.

Observe the cell substance well coloured, and hardly at all shrunken, the nuclei deeply coloured and having a sharp outline, the matrix coloured very slightly.

3. Transverse sections of a cartilaginous rib (picric acid)¹ of a fully grown, but not old, animal. Stain one section in picrocarmine for $\frac{1}{2}$ hour to 1 hour, and another in acid hæmatoxylin for 15 to 20 minutes. Mount the former in glycerine, and the latter in balsam.

Observe that the cells are arranged in groups (each group having arisen by division from a single cartilage cell), note the outline of the thin layer of newer cartilage (capsule) around each cell, sometimes the whole of

¹ The fixing agent in which the tissue is placed on being removed from the body is here and later given in brackets. The details of treatment are given in the Appendix.

the cells in a group may be seen to be also surrounded by a thin layer just marked off from the rest of the matrix. Towards the outside of the cartilage the cells become flattened in a direction parallel with the surface.

4. Take a piece of costal cartilage from a recently killed oldish animal; cut thin transverse sections either free hand or with a freezing microtome and transfer them with a brush to a watch-glass containing normal salt solution. Mount one section in normal salt solution. Place another in two or three drops of osmic acid .5 p.c. in a watch-glass and cover it up.

a. In the former observe under a high power, that

The cells frequently show signs of partial degeneration, containing fat globules which may be nearly as large as the cell; these are very highly refractive and so have a very distinct outline.

In places the matrix may be fibrillated and towards the centre of the section there may be a nodular semi-opaque deposit of lime salts. Irrigate with 1 p.c. hydrochloric acid; the calcareous deposit is dissolved, but the fibrillation is not affected (it differs thus from the fibrillation of white fibrous connective tissue, *cp.* Lesson VII. § 2).

b. Mount in dilute glycerine, and examine the section which has been treated with osmic acid; the fat globules are stained a deep brown-black, the cell substance, the nuclei and the matrix are but slightly stained. On keeping, the fat globules become black.

5. Sections of cartilage from the head of a cuttlefish (picric acid). Stain a section with acid hæmatoxylin, wash it, and mount in glycerine. If it is too deeply stained, place it in a little 1 p.c. acetic acid and when it is sufficiently decolorised, wash out the acid. Observe

a. The groups of cartilage cells.

b. The marked **processes** proceeding from some of the cells. Sometimes the processes of neighbouring groups may be seen to join.

6. Take a small piece of the ear of a freshly killed mouse or young rat, remove the skin and scrape away the tissue surrounding the ear-cartilage. Mount the cartilage in normal saline solution.

Note the cartilage cells closely packed together, most have one or more refractive fat globules in them. (The fat increases up to a certain limit with the age of the animal.) Unmount, stain the specimens with osmic acid.

DEMONSTRATION.

Transverse section of ear of adult rat. (Alcohol, acid hæmatoxylin, or gold chloride and formic acid.) Note the **parenchymatous cartilage**. The cell-spaces, generally polygonal, are separated by very narrow partitions of matrix. The spaces contain very little cell substance.

LESSON VII.

CONNECTIVE TISSUE.

1. **Elastic Tissue.** Tease out in water a morsel of ligamentum nuchæ, *e.g.* of ox. It is almost entirely made up of rather large, branching, and anastomosing fibres having distinct outlines, and curling at their ends.

Irrigate with acetic acid (1 to 5 p.c.); the **elastic fibres** are unaffected; the small amount of white fibrous tissue present, swells up; a few nuclei only come into view.

2. **Tendon.** Cut off the tip of the tail of a mouse or young rat; cut through the skin for a short distance, break off a tail vertebra, and pull straight outwards. A bundle of small tendons will be obtained. (A similar bundle can be obtained in the same way from each vertebra.) Gently stretch this over a glass slide from edge to edge, and hold it in this position till the edges are dry, touching the mid portion with a brush moistened with normal saline solution; the fibres are thus kept extended. Cover with a cover-slip. Note the **white fibres** consisting of parallel bundles

of wavy fibrillæ. Slowly irrigate with acetic acid 1 p.c., *watching the while* under a high power. Between the bundles of fibrillæ rows of cells will become visible. Note in each the round or oval nucleus, the rectangular outline of the cell, and the finely granular cell-substance. Indications of flange-like lateral processes may be seen as lines running along the cells in the direction of the tendon. After the acetic acid has been added for some little time the cell substance becomes very indistinct, and between the swollen bundles scarcely anything is seen but rows of elongated shrunken nuclei. (Cp. Action of acetic acid on cartilage cells, Less. VI. § 1 b.)

3. Place a piece of tendon (see § 2) in a saturated aqueous solution of picric acid for a day. Wash with water. Stain deeply with picrocarmine. Wash with water. Take a small piece of the tendon, tease one end carefully, without adding fluid, using very fine needles; add a drop of water, cover, and examine with a high power. Note the very small fibrillæ, now more or less separated from one another.

4. Pull out another bundle of tendons: place them in .2 p.c. nitrate of silver for 5 to 10 minutes. Remove to water, move them to and fro once or twice, and gently separate them, renew the water and expose to light, now and then turning them over. In about half-an-hour pass a tendon through alcohols to clove oil. Place on a slide and examine with a low power. The *layer of epithelioid cells* covering the tendon, will in places be seen (some are often rubbed off in pulling out the tendon from the tail). Cut out the successful pieces, mount in balsam, and examine with a high power.

5. Transverse sections of a rather large tendon¹ such as that of the digastric muscle of a cat or rabbit.

¹ A tendon may be fixed by placing it fresh in 30 p.c. alcohol for a few hours, and then in 75 p.c. alcohol; or by placing it in a saturated aqueous solution of corrosive sublimate for a day.

Stain and mount in balsam. Note the connective tissue sheath around the tendon, the septa from the sheath dividing the tendon into bundles, the branching **tendon-cells** between the white fibres of the tendon.

6. **Branched connective tissue cells of cornea.** Take a pithed frog, cut away the nictitating membrane, squeeze the side of the head to make the eye bulge out, then slice boldly at the edge of the cornea; take up its edge with forceps, and with fine scissors cut through the attached part at its junction with the sclerotic; any blood which may be on the cornea should be removed by placing it in a watch-glass containing normal saline solution and very gently brushing it. Put it in gold chloride .5 p.c. solution, gently extend it into its normal shape with two finely pointed glass-rods, leave it for thirty to forty minutes, *wash well with water*, transfer to about 20 c.c. of water just acidulated with acetic acid, and expose to light. When the cornea has become of a red- or blue-violet colour¹, put it on a slide with glycerine, and carefully scrape both surfaces to remove the epithelium, examine it under a low power to see that the epithelium is removed, when it is, mount in formic glycerine and examine it under a high power. If the cornea does not become properly stained in a day, place it in a test-tube with a saturated or nearly saturated solution of tartaric acid, and heat for a few minutes to a temperature at which the test-tube can barely be held in the palm of the hand. This will probably complete the reduction.

¹ Probably it will not be stained until the following day.

Note the stained connective tissue corpuscles, with numerous fine branching processes which anastomose with the similar processes of neighbouring cells. This preparation should be preserved for examination of the nerves of the cornea (Lesson XIV. § 11).

Branched connective tissue cells are well seen in the tail of the tadpole (Perenyi's fluid) stained with picrocarmine. The surface epithelium is removed by brushing or partial teasing. The preparation may also show a stage in the development of capillaries.

7. **Areolar Tissue.** Cut transversely through the skin of the back of a recently killed frog. Turn the lower piece of skin downwards, cutting it through at the sides. Note the connective tissue stretching from the skin to the body at the level of the posterior end of the urostyle.

Using fine-pointed scissors and forceps, cut out on one side this piece of connective tissue. Shake it in normal salt solution to wash away blood; touch blotting-paper with it to remove excess of fluid, and put it on a dry slide. Spread out the piece of tissue with needles; drawing out gently the edges all round till they adhere to the slide. As soon as it remains extended, put on a cover-slip, and lightly press it down; this must be done before the tissue becomes dry.

The tissue is chiefly composed of white fibres having a wavy course, and more or less distinct fibrillation; the bundles are of unequal size and run across one another in all directions. Some small elastic fibres running singly will also be seen, their outlines are more distinct than those of the bundles of white fibres; they branch and anastomose freely with one another. Where the film is well stretched the elastic fibres run

for the most part quite straight, elsewhere they are curled at their ends and have a more or less sinuous course. Irrigate with acetic acid, the elastic network then becomes distinct.

(The stretching of a piece of tissue on a slide or cover-slip as above, so that it adheres but is not allowed to dry, will be spoken of as making a **moist film**. It is often simpler to extend the tissue by smoothing it outwards with the fingers, instead of using needles. The film may be fixed by adding a few drops of 95 p.c. alcohol, and leaving it for half-an-hour, covered up. This will be spoken of as **fixing a moist film with alcohol**. The film may then be stained.)

8. *Inter-muscular connective tissue of frog.* Make a complete transverse incision of the skin of a pithed frog, in the abdominal region. Tear the skin backwards so as to expose the thigh muscles. With forceps lift up the muscles of the ventral surface of the thigh near the knee, and cut away all the muscles over the femur. Place the muscular mass on cork, deep surface uppermost, and gently pull laterally the muscles on either side of the sartorius, a thin layer of connective tissue on either side of the sartorius will come into view. Pin out the muscles so that the connective tissue layer is stretched, wash it with salt solution to remove adhering blood, pour over it 95 p.c. alcohol and leave for half-an-hour. Then cut out the connective tissue and stain it with hæmatoxylin and eosin.

The connective tissue fibres will be stained with eosin, the nuclei of the cells with hæmatoxylin.

A similar preparation may be made of the connective tissue between the muscles of the rat or rabbit. In this case, the piece of tissue between the muscles may be picked up with forceps, cut out, and spread out to make a moist film in the manner given in § 7. When the edges are dry, the tissue is fixed with alcohol.

9. Dissect back a piece of skin of a recently killed young rat, and into the subcutaneous tissue attached to this inject a .5 p.c. solution of gold chloride with a Pravatz syringe, until the fluid begins to exude from the small bullæ of tissue so raised. After a few minutes snip off with a sharp pair of scissors one or two pieces as thin as possible, and place them in a watch-glass containing 25 p.c. formic acid¹. When the pieces are well coloured, which will probably be in two or three hours, shake them gently in a glass containing distilled water. Spread out a piece on a slide till the edges adhere (cp. § 6), put a small drop of formic glycerine in the centre of a cover-slip and lower it on the preparation and press gently. The preparation will improve in a day or two. Observe

a. The usually angular leucocytes scattered about between the bundle of fibres; the fibrous bundles will be more or less swollen up by the formic acid, and so not offer sharp outlines.

b. Connective tissue corpuscles rather larger than the leucocytes, and having processes which, in some cases, are seen to be connected with the processes of other similar cells. They are however swollen and indistinct.

c. Larger, more rectangular cells, with coarsely granular cell substance and oval nuclei; they are often in rows especially by the blood vessels; on a side view these appear as thin, long cells. These are sometimes called *plasma cells*.

This preparation should be preserved for the observation of fat tissue.

10. Make on a cover-slip a moist film of connective tissue from a rat (cp. § 7). Hold it for a minute in methylene blue dissolved in 75 p.c. spirit (cp. Less. I., § 14), dip it in 75 p.c. spirit, and at once press lightly between sheets of blotting-paper. Put it aside to dry. When dry, add a drop of balsam and mount. Note that the coarse granules of the cells mentioned in § 8 c. stain deeply with methylene blue.

¹ Formic acid 1.06 sp. gr.

11. Snip off a small piece of the subcutaneous tissue in a foetal or new-born mammal¹.

Spread it out on a slide till the edges stick, then (a) fix with alcohol and stain with hæmatoxylin and eosin, or (b) treat .5 p.c. gold chloride on the slide, for 30 to 45 minutes, wash, reduce with 25 p.c. formic acid, or (c) add a drop or two of 1 p.c. aqueous solution of Spiller's purple or methylene blue, leave for a minute or two, wash with water, and mount in water.

Note the cells of various shapes, for the most part with faint outlines, but with distinct and rather large nuclei; irrigate with dilute acetic acid, watching closely the early changes produced.

This may also serve to show the development of fat-cells.

12. Note in the section of costal cartilage prepared in Lesson VI. § 6 the connective tissue layer outside and closely attached to it forming the perichondrium; in places sections of tendons running into the cartilage will probably be seen.

13. **Pigment cells.** Pin out on a frog-board one of the webs between the toes of the frog used in § 6, and observe first under a low and then under a high power. There will be seen large corpuscles loaded with dark pigment, and possessing numerous branched processes. In some places the pigmented cells will appear as round dots, the processes in this case having been retracted. Every intermediate stage between these two states may be observed by watching from time to time.

Cut off the foot, and pin out on cork the web between two of the toes. Pour a little 95 p.c. alcohol

¹ An animal just killed serves best, but one preserved in picric acid or Müller's fluid will answer the purpose; if it is desired to observe the forming fat-cells the animal must not have been transferred to alcohol stronger than 50 0/0.

on the foot. In about 5 minutes cut out the web, and put it in 95 p.c. alcohol in a watch-glass. In a quarter of an hour transfer to fresh 95 p.c. alcohol for another quarter of an hour. Then remove excess of alcohol by pressing the web for a moment with dry blotting-paper, and place in oil of cloves. When the web is transparent, mount in balsam; and examine again the pigment-cells.

Pigment-cells will be seen later in preparations of the mesentery, and of the *lymphatica cisterna magna* (Lesson XXV. §§ 1, 2).

14. **Fat-cells.** Cut out a small piece of the omentum, from a part containing comparatively little fat. Spread it out on a slide and mount it in normal saline solution. Observe

a. Under a low power, the groups of highly refractive fat-cells.

b. Under a high power, the variable size of the fat-cells, the apparent absence of a nucleus, the connective tissue passing between and over the cells.

15. Place a similar piece of omentum in osmic acid .5 p.c. for about half-an-hour, wash with water, cut in half, mount one half at once in dilute glycerine, and the other half, after staining it for thirty minutes in picrocarmine. Observe that the fat-cells are stained a deep brown-black (cp. Less. VI. § 4, b). If the tissue before being mounted be kept in alcohol for a day the tint of the fat globules will become deep black.

16. Take a small piece of omentum, which has been kept for some time in alcohol, stain it deeply with

hæmatoxylin, pass through the alcohols to turpentine or xylol and leave for a quarter of an hour. Mount in balsam.

Observe the groups of shrunken cells from which the fat has been thus removed. Note the small amount of cell substance forming the boundary of a cell, and the flattened deeply stained nucleus in the cell substance.

17. In the gold chloride preparation of subcutaneous tissue made above, § 9, observe

The network of capillaries in a small collection of fat-cells. In some fat-cells a nucleus and a small amount of protoplasm surrounding the fat may be seen.

DEMONSTRATIONS.

1. Fibrillæ of tendon under a high power, cp. § 3.
2. Epithelioid cells covering tendon, cp. § 4.
3. Gold chloride preparation to show cells of areolar connective tissue of rat, cp. § 9.
4. Film preparation of connective tissue stained with methylene blue, cp. § 10.
5. Section of umbilical cord to show mucous connective tissue.

LESSON VIII.

FIBRO-CARTILAGE. ELASTIC CARTILAGE. BONE. TEETH.

1. **Fibro-cartilage.** Sections of semi-lunar cartilage of knee joint of dog (picric acid; cut frozen) cut parallel with the mass of the fibres. Stain one section with picrocarmine and mount in formic glycerine, stain another in hæmatoxylin and mount in balsam. Note

The bundles of white fibrous tissue.

The rather large cartilage cells lying here and there between the bundles of fibres, and usually in rows; each is surrounded by a capsule consisting of a small amount of hyaline cartilage.

2. Sections of intervertebral disc¹ with adjoining faces of the vertebræ, cut at right angles to the faces of the disc. Stain with hæmatoxylin or picrocarmine. Note,

The intervertebral disc, consisting partly of fibro-cartilage, partly of ligamentous tissue. The bundles cross, chiefly in two directions at right angles to, and parallel to, the faces of the disc.

¹ Rabbit. Remove the spinal cord. Take several successive vertebræ. Saw through them about 3 mm., from the intervertebral disc. Place the discs with the bone attached, in a hardening and decalcifying fluid (cp. App. p. 364).

The fibro-cartilage passing on the faces of the disc either into a thin layer of hyaline cartilage or directly into the decalcified bone, and on its surface into fibrous connective tissue.

A thin layer of spongy bone, the spaces containing red marrow with a considerable number of fat-cells.

A little farther from the disc, a layer of cartilage, with cells in rows as if ossifying; and a little farther still, spongy bone with red marrow and fat-cells.

3. Elastic Cartilage and transition to Hyaline Cartilage. Vertical sections of sheep's arytenoid cartilage¹ (picric acid). Stain (*a*) one section with picrocarmine, and another (*b*) first with hæmatoxylin and then with dilute aqueous picric acid; mount in glycerine.

Examine (h. p.), proceeding from the deeper part of the section to the free edge. Observe—

In (*a*) the hyaline cartilage, showing at its upper border, fine granular fibres—the elastic fibres—in the matrix. These rapidly increase, till the matrix consists largely of a close meshwork of fibres, leaving a hyaline portion immediately around the cells. In places, the elastic substance may take the form of thick nodular bars.

Externally, areolar tissue; note the transition of the elastic cartilage into this by the elongation of the cells, the disappearance of the capsules, and the substitution of white fibrous tissue (stained red) for elastic fibres.

In (*b*) the elastic meshwork stained brilliant yellow, and the hyaline substance around the cartilage cells

¹ In the sheep the deeper part of the arytenoid cartilage is hyaline. The cartilage is cut through this, the apex cut off and the mucous membrane removed. The epiglottis may be taken instead of the arytenoid, but it does not contain hyaline cartilage.

stained blue. The hyaline substance, being stained, appears to be much more abundant than in (*a*). Here and there a patch may be seen with very little blue stained substance around the cells, this is elastic fibro-cartilage.

4. Structure of Bone. Transverse section through the shaft of a long bone. (The bone is dried; thin pieces are cut with a saw, ground down and mounted in balsam.) *a.* Examine under a low power. Most of the smaller spaces (*i.e.* Haversian canals, lacunæ, canaliculi) will be filled with air or débris and will therefore appear dark. Observe

The central cavity surrounded by a small amount of spongy bone with rather large irregular spaces, Haversian spaces; externally the spongy bone passes into the compact bone: note the transition from the Haversian spaces to the Haversian canals.

In the compact bone the Haversian systems, each consisting of a **Haversian canal** surrounded by concentric **lamellæ**, which are chiefly marked off from one another by the **lacunæ** which lie between them.

The intersystemic lamellæ between the Haversian systems; in the outer part of the bone these run for the most part parallel with the surface (circumferential lamellæ).

In the spongy bone the lamellæ run in the main concentric with the spaces.

b. Examine under a high power. Observe

The irregularly fusiform lacunæ giving off numerous wavy branches, the **canaliculi**, which run across the lamellæ to join the similar branches of neighbouring

lacunæ. In addition to this connection, canaliculi open into the Haversian canals in the compact bone, into the Haversian spaces and central cavity in the spongy bone.

5. Longitudinal section of the shaft of a long bone. (Prepared as in § 4.)

a. Examine with a low power. Observe

The Haversian canals running in the main parallel with the surface of the bone, they have connecting branches and open here and there on the surface and into the central spaces.

The lamellæ running for the most part parallel with the Haversian canals. Where a Haversian system is cut obliquely the lamellæ are concentric with the canal.

b. Examine under a high power. The individual lacunæ are much as in the transverse section, but rather longer; observe the arrangement of the canaliculi (cp. § 4, *b*).

6. Transverse sections (in paraffin) through the shaft of a decalcified and stained long bone¹, as femur of rat. Mount in balsam. Note

The periosteum formed of connective tissue closely attached to the bone.

In the bone, each lacuna is occupied by a cell, the **bone corpuscle**, probably shrunken from the walls of the lacuna. The canaliculi are scarcely visible.

¹ For methods of decalcification cp. Appendix. The tissue may be stained in bulk with picrocarmine, but it is better to stain the sections on the slide (Less. iv. § 13).

The Haversian canals are chiefly occupied by small blood vessels.

The **white marrow**, consisting mainly of fat-cells (cp. Less. VII. § 14). Small clumps of marrow cells are seen, most of them are either leucocytes or resemble leucocytes, and here and there **giant cells**, large cells with irregularly lobed nucleus.

7. Remove the periosteum from a decalcified parietal bone (nitric acid); tear off from the surface thin strips of the bone matrix and mount them with the inner side uppermost in water. Under a high power note the *perforating fibres* projecting from the surface and the apertures through which similar fibres have passed. Examine carefully the thinnest part of the strip (containing fewest lamellæ) for the fine *decussating fibres* of the matrix; add acetic acid, both the perforating and decussating fibres swell up and become indistinct or lost to view.

8. **Ossification.** Longitudinal sections through the head of the femur of a newly-born (or foetal) cat or rabbit (picric or chromic acid). Stain with eosin or picrocarmine, mount in balsam. Observe, passing from the head to the shaft

The normal hyaline cartilage.

The longitudinal rows of cartilage cells; many of the cells are triangular in section. The layer of large, probably shrunken, cartilage cells; they are also in rows and have only a small amount of matrix around them.

The irregular spaces (secondary areolæ) of the primary endochondral bone. The bony trabeculæ may show a thin central portion of decalcified cartilage less stained with carmine than the osseous tissue, but the

distinction will probably not be very obvious¹. Covering the trabeculae and in the spaces are **osteoblasts**, cells larger than ordinary leucocytes, with a single ovoid or spherical nucleus, and finely granular cell substance. The spaces also contain blood vessels and some jelly like connective tissue.

Osteoclasts may be seen here and there in contact with the bone matrix; they are large multinuclear cells, and where they touch the bone are often striated at right angles to the surface.

Note on the outside of the bone below the head, the periosteum, and the thin layer of spongy periosteal bone beneath it.

9. Transverse section of the shaft of the bone used in § 8. Note

The spongy periosteal bone beneath the periosteum, the osteoblasts especially numerous in the lower layer of the periosteum, and in the peripheral bone spaces. Many of the osteoblasts are elongated and their smaller ends appear to run into the matrix.

The spongy endochondral bone in the centre; there may be the beginning of a central space but no fat-cells are present.

10. **Structure of Teeth.** Longitudinal sections of teeth prepared as bone, § 4. *a.* Examine under a low power. Observe the **dentine** surrounding the pulp-cavity, the **cement** or **crusta petrosa** covering the dentine of the fangs, and the **enamel** covering the

¹ The two parts are very distinct in sections stained in the Ehrlich-Biondi mixture.

dentine of the crown. Note the general arrangement of the dentinal tubules.

b. Examine under a high power, and study in detail

The **Dentine**. In the matrix, apparently homogeneous, are numerous dentinal tubules which run in a wavy course from the pulp-cavity outwards. These, dividing as they go, and giving off many anastomosing lateral branches, finally end either in loops or in small irregular cavities, the so-called interglobular spaces, on the surface of the dentine.

In some places the dentinal tubules are cut transversely. Here the central dark spot indicates the space formerly occupied by the dentinal fibre, the ring round this is the dentinal sheath.

The **Cement** or **Crusta Petrosa**. This differs little from bone, but Haversian canals are generally absent.

The canaliculi of the lacunæ next to the interglobular spaces, open into these, thus bringing the dentinal fibres into connection with the bone-corpuscles.

Where the cement is massive, wavy 'contour' lines may be seen, indicating the successive deposits.

The **Enamel**, consisting of striated fibres or prisms, perpendicular to the surface of the dentine.

11. Sections of lower jaw of a foetal mammal (chromic acid 0.2 p.c.). Stain with picocarmine. Note the stages of the development of the teeth, and also the osteoblasts and osteoclasts of the young sub-maxillary bone.

DEMONSTRATIONS.

1. Transverse section of rib to show red marrow¹. Note the numerous marrow cells, the scattered fat-cells and occasional giant cell.

2. Transverse sections of teeth through the crista petrosa and through the crown. Compare with the longitudinal sections described in the text.

3. Longitudinal section of decalcified tooth, showing the pulp.

¹ Short piece of rib of growing animal, fixed in mercuric chloride; decalcified; imbedded; stained on the slide.

LESSON IX.

STRUCTURE OF CONTRACTILE TISSUES.

1. Ciliated Cells. Cut off the head of a recently pithed frog, and cut away the lower jaw. With fine forceps and scissors, cut out on one side a piece of the mucous membrane of the roof of the mouth and place this on a slide, mucous surface uppermost. Pour 2 or 3 drops of .5 p.c. osmic acid on the remainder of the mucous membrane of the roof, cover it with a watch-glass for 15 to 20 min. (cp. § 1, *b*).

a. To the piece of membrane on the slide add a drop of .6 p.c. sodium chloride; scrape the epithelium from a portion of the membrane and tease some of the scrapings in the salt solution. Place a fragment of paper at the edge of the drop; and cover. Note

The shimmering appearance caused by the movements of the cilia in the larger portions.

The movements of the cilia in the isolated cells or clumps of cells. Observe carefully the cilia which are moving slowly; it may be seen that the down-stroke (contraction) takes place more quickly than the return

(relaxation); there is no perceptible pause between the two movements.

The results of ciliary action. Granules and blood-corpuscles are driven along; detached cells may also be seen carried about by the action of their own cilia.

The contracted, almost globular form of the isolated cells.

b. Remove from osmic acid to water; cut out a piece of the mucous membrane, wash it gently with water, place it in picrocarmine for $\frac{1}{2}$ hour or more, wash, scrape off a little of the epithelium, transfer to a small drop of water faintly coloured with picric acid, tease, cover, and examine (h. p.). Note

The patches of epithelium consisting of ciliated cells and of swollen mucous (goblet) cells. Viewed from the side, adjoining ciliated cells are seen nearly to cover in at the surface the mucous cell between them. Viewed from the surface, numerous, closely set, rather faint dots represent the cilia, here and there are seen the small round openings of the mucous cells, at a lower focus the swollen portions of the mucous cells come into view, surrounded by narrow stained bands, formed of the bodies of the ciliated cells.

The isolated ciliated cells. (If the isolation is not good, tap the cover-slip.) The cilia are densely studded over the whole surface. Beneath the cilia is a hyaline or faintly striated layer, the **hyaline border**. A little below this, the cell generally tapers, cp. the shape with that seen in *a*. The stained nuclei are conspicuous.

The preparation may be preserved by adding a drop of dilute glycerine to the edge of the cover-slip so that

it gradually takes the place of the water, but the cilia will then be less obvious.

2. **Striated muscle of frog.** Cut through the skin of the front of the thigh of the frog used in §1, note the band-like sartorius muscle running somewhat obliquely across the thigh from the pelvis to the knee, seize with forceps the connective tissue lying along each border and tear it away from upper to lower end.

Take up with fine forceps a few fibres at one end of the muscle, and gently pull them out to the opposite end. Stretch out the bundle on a dry slide, separate the fibres a little in the centre, place a bristle across them and press gently: remove the bristle, and cover.

a. Note (h. p.) the fairly transparent muscular fibres and their varying size.

The transverse striation; alternate dim and light bands passing through the whole thickness of the fibre.

Longitudinal granular lines or rows of small granules varying greatly in number and distinctness in different fibres.

b. Add a drop of normal salt solution. The longitudinal granular lines will become more distinct. Some of the fibres may show a distinct longitudinal as well as a transverse striation.

Where the bristle was pressed on the fibres, the muscular substance will in some cases be broken, leaving a delicate structureless sheath, the **sarcolemma**, stretching across the gap; possibly in other places, as where a fibre is bent, the sarcolemma may be seen as a fine bulging line.

c. Irrigate with 1 p.c. acetic acid; after a time the shrunken elongated nuclei of the fibres come into view scattered throughout the muscle substance.

3. Tear off a very small strip of frog's muscle (extended in alcohol) including one tendinous ending, and tease out as finely as possible in dilute glycerine. Note

The transverse striation is more distinct than in § 2. Sometimes a very thin dark line may be seen in the middle of the light one.

A longitudinal striation of the fibres is more or less marked, and some of the fibres have been split up into very fine transversely striated threads.

At the tendinous end, the abrupt ending of the muscle fibre is seen, and the continuation of the sarcolemma into connective tissue, cp. with § 4¹.

4. Cut through the skin in the mid ventral line in a small frog, lift up the flap of skin on one side over the middle of the sternum, a thin band of muscle, the *sterno-cutaneous muscle* (m. cutaneus pectoris), will be seen running from the skin downwards towards the lower part of the sternum. Cut through the skin above and below the muscle, and keeping it well stretched, pour a little .5 p.c. osmic acid over it. In a minute or so the muscle will be fixed; the connective tissue surrounding the muscle may be torn away with

¹ The fibres are readily isolated and the passage of the sarcolemma into the tendon seen in muscle treated as follows. A pithed frog is placed in water at 50° C. to 55° C., allowed to cool, and the muscles preserved in 70 p.c. alcohol. The nuclei may be stained with picrocarmine.

fine forceps (but in doing this there is considerable danger of injuring the muscle and its nerve); cut it out, being careful to cut the upper end as close as possible to the skin; place it in osmic acid for 15 to 30 minutes, wash with water. Divide the muscle transversely near its upper end. Mount in glycerine, the upper end having the outer surface, and the lower end the inner surface uppermost (the latter will be required in Less. XIV. § 10).

Observe at the upper end of the muscle the endings of the muscular fibres, these will probably be covered by a good deal of connective tissue, but the rounded or conical ends of the muscle-substance of the fibres and the continuation of the sarcolemma into the connective tissue (tendon) will be seen.

The muscle, after it has been treated with osmic acid and washed, may be stained with picrocarmine; or the muscle of the opposite side may be fixed with alcohol (Less. VII. § 13) and stained with picrocarmine; thus the nuclei will be brought out. The nerve fibres stain better if the muscle after osmic acid is placed for a day in 30 p.c. alcohol.

5. **Striated muscle of insect.** Remove with as little injury as possible one of the thin muscles from the leg of *Hydrophilus*¹, tease it out a little without adding fluid, and cover. The fibres will for a brief period be seen in a normal condition. In many, the alternate dim and bright striæ will be very distinct; in others the transverse markings will be more or less obscured by an appearance of longitudinal fibrillation. Occasionally fibres are met with, having only a confused

¹ If not obtainable, take the large common water-beetle (*Dytiscus marginalis*), or failing that a cockroach or a fly.

granular aspect. If the preparation has been successfully made, waves of contraction may, at times, be seen to travel along the fibres.

Observe in the fibres with distinct transverse striation, that,

The dim band has a faint longitudinal striation, as if made up of small rods.

The bright band shows a dotted line running across its centre.

The usually round nuclei are imbedded in a granular mass of protoplasm (sarcoplasm); the whole forms a band or rod running a variable distance in the substance of the fibre. If the nuclei are not distinct, irrigate with acetic acid.

6. Tease out a little of the wing (thoracic) muscle of an insect, note the *sarcostyles*, and the considerable amount of sarcoplasm. The sarcostyles are small, but much larger than the fibrillæ seen in § 2. Measure one or two.

7. Sections of a piece of wing muscle from an insect (Flemming's fluid). Stain on a cover-slip (cp. Less. iv. § 7). Note the round areas of the sarcostyles imbedded in the granular protoplasm.

8. **Unstriated muscle.** Remove the stomach of the frog used in § 4. Cut it open, and pin it out. Take up the edge of the mucous membrane with forceps, partly tear and partly cut it away from the muscular coat. Place a piece of the muscular coat about 3 mm. square in a watch-glass with 35 p.c. potassium hydrate¹.

¹ Potassium hydrate readily isolates unstriated muscle cells, but it makes them more homogeneous looking than they are in life. The nucleus and the faintly granular protoplasm around it are better seen

In 20 to 30 minutes, tease the piece in the watch-glass into smallish fragments, remove one or two of these to a slide,—tease further and cover. Note

The isolated muscle cells, long cells tapering at the ends, in some the nucleus—here pale and watery looking—will be seen in the mid and broader region.

The tapering ends of the cells projecting from the less teased fragments of tissue.

9. Cut out one half of the bladder of a pithed frog; put it in normal salt solution and cut it open. Make a moist film preparation, mucous membrane uppermost (Less. VII. § 7), rubbing it gently with the finger to remove the epithelium. Fix with alcohol. Press with blotting-paper, add hæmatoxylin at once. When it is rather deeply stained, wash, blot again, and add eosin in 75 p.c. alcohol. In about a minute, pour off the eosin, wash with 95 p.c. alcohol; blot, at once add clove oil. Mount in balsam.

Note the plexus of unstriated muscle cells.

10. **Cardiac muscle.** Treat a small piece of the ventricle of just killed rat or mouse with 35 p.c. potassium hydrate¹ by the method given in § 8. In the teased specimen, note

The isolated muscle cells; they are short, thin columnar cells, many having a short process coming off obliquely from the body of the cell. At about the centre of each cell is a nucleus. The transverse striation is not very distinct. The cells have no sarcolemma.

by the following method. A small strip of unstriated muscular tissue is stretched and placed in potassium bichromate .1 p.c. or in 30 p.c. alcohol for 2 to 3 days, washed with water, stained with picocarmine and mounted in dilute glycerine.

¹ See Footnote on p. 75.

11. Isolate the cardiac muscle cells of the frog by the method given in § 8.

Note that the cells generally resemble unstriated muscle cells in shape, but are much shorter and are transversely striated. By this method the striation is not marked.

Some cells may be seen with branches or with flattened expansions.

12. *Examination of muscle with polarized light.* Place one Nichol's prism under the stage of the microscope in the position of the condenser. Select a specimen of striated muscle, in which the fibres are isolated, and the planes of the discs at right angles to the stage. Using an ordinary ocular, focus the piece of muscle to be observed.

Substitute for the ordinary ocular the frame containing an ocular with a Nichol's prism (the analyser) above it. Put the pointer of the analyser at 0 of the scale on the frame. Turn the frame with the analyser round one complete circle. The field during the complete revolution becomes dark twice and light twice.

Turn the frame so that the field is at its maximum brightness, and clamp the frame. Focus the muscle. Turn the analyser slowly through 90° , note that at 45° the thick discs are light, *i.e.* they are doubly refractive; whilst the rest of the fibre more or less completely disappears.

Turn the specimen on the stage through an angle of 45° to see if any other part of the fibre becomes visible. (If the principal plane of a doubly refracting substance is parallel to the principal plane of either prism, no light is transmitted.)

The disposition and amount of the singly and doubly refractive substance vary with the nature of the muscle and with its condition, whether fresh or hardened.

DEMONSTRATIONS.

1. Transverse sections of mammalian striated muscle (chromic acid .5 p.c.). Observe

The connective tissue (perimysium) around the

bundles of fibres and around the whole muscle; from this runs a small amount of connective tissue (endomysium) between the muscle fibres.

The cut ends of the fibres are finely dotted, corresponding with the fibrillæ.

The nuclei lie just beneath the sarcolemma, few or none being imbedded in the muscular substance of the fibres (cp. § 5, c).

2. Longitudinal section of mammalian striated muscle (alcohol, picrocarmine) to show the nuclei of the fibres.

3. Teased striated muscle fibres (picric acid) to show the splitting of the fibres into discs.

4. Striated muscle fibres under a polarising microscope (cp. § 12).

5. Longitudinal section of the intestine of cat or dog at right angles to the surface. (Alcohol or .2 p.c. chromic acid. Hæmatoxylin.) In the longitudinal coat, note the sheet of muscle cells with elongated stained nuclei; in the circular coat, which is cut transversely, note the bundles of muscle cells; the nuclei are only seen in some of the cells; they appear as round, deeply stained central spots.

6. Section of ventricle of a small mammal (alcohol; hæmatoxylin). Note the meshwork formed by the junction of the cells, and the hyaline cement substance at the junctions.

LESSON X.

CHEMISTRY OF MUSCLE. DERIVED ALBUMINS. ALBUMINOIDS. EGG-WHITE.

1. **Myosin.** Strip off the muscles from a rabbit or other animal and chop them up as finely as possible, place them in a large jar filled with water and stir; in about a quarter of an hour place a piece of muslin over the top of the jar and pour off the water; fill up the jar again with water and let it stand for an hour, then pour off the water and refill the jar as before. When this has been repeated once or twice the greater part of the substances soluble in water will have been removed. The filtrate from a small quantity of muscle which has been in an equal bulk of water for an hour should give no proteid reaction if the muscle has previously been thoroughly washed.

Collect the muscle on linen, squeeze out the water, grind it up with clean sand and add 5 times its bulk of 10 p.c. ammonium or sodium chloride, and stirring occasionally place it aside for an hour or so (naturally if it is left longer more myosin will be obtained). Filter through muslin, through linen, and then through

coarse filter paper. A somewhat viscid fluid is obtained; pour this into a tall vessel containing about a litre of water and observe the precipitate of **myosin** which takes place (or the fluid as it filters may be allowed to drop into water).

After a short time decant or draw off with a pipette as much fluid as possible, shake up the rest and pour about 5 c.c. with the myosin suspended in it into each of three test-tubes.

a. Add drop by drop a strong (*e.g.* 20 p.c.) salt solution, the precipitate soon dissolves; then place it in a water bath at about 50° C. with a thermometer and heat; at about 57° C. the fluid becomes milky (or a precipitate is formed) owing to the coagulation of the myosin.

b. Add powdered salt, the precipitate soon dissolves, but when the fluid is saturated with salt is reprecipitated (the precipitate may be in the form of fibrous-looking clumps).

c. Dissolve the suspended precipitate in salt solution, and test for proteids, *e.g.* with xanthoproteic reaction (cp. Less. III. § 4).

Myosin belongs to the class of **globulins**; it will have been seen that it is insoluble in water and in saturated neutral salt solutions, soluble in not too dilute solution of neutral salts, its solution coagulating on boiling (cp. also Less. III. §§ 6, 15).

2. *Muscle-plasma.* Wash the blood out of the blood vessels of a frog or, better, out of the limbs of a just killed mammal, with cold normal salt solution (5° C.). When the muscles are more or less completely freed from blood, throw them into a freezing

mixture. When frozen chop them up. The fluid which can be expressed from the fragments as the temperature rises is muscle-plasma. This may be obtained more easily in a diluted form by extracting the muscle fragments with a little cooled 10% NaCl solution, or half saturated Na_2SO_4 solution.

a. To dilute salted muscle-plasma add three times its volume of water and set it in the warm bath. In half-an-hour the fluid will usually have clotted, and myosin be formed.

b. Determine the coagulating points of the proteids present in dilute salted muscle-plasma. A small coagulation occurs at 47° C. (paramyosinogen), a large coagulation at 56° C. (myosinogen), and if this is filtered off, further small coagulations may be obtained at 63° (myoglobulin) and at 73° (albumin).

Derived albumins.

3. Acid Albumin or Syntonin. Treat a portion of muscle washed as in § 1 with ten to twenty times its bulk of HCl .2 p.c. and place in the warm chamber at about 40° C., frequently shaking. After three or four hours most of the myosin of the muscle will have been converted into **acid-albumin** and dissolved. Acid albumin may be prepared in a similar way from white of egg or from serum.

Filter and carefully neutralize the filtrate with a weak solution of sodium carbonate, a copious bulky precipitate of acid albumin takes place; if too much alkaline salt is added the acid albumin will be converted into alkali albumin and redissolved (cp. § 4). Filter and wash the precipitate once on the filter with water, then break through the filter paper and wash the precipitate into a beaker with water. The acid albumin is

thus obtained suspended in water. Place a little in each of two test-tubes.

a. Add a little HCl $\cdot 2$ p.c., the precipitate at once dissolves and is not precipitated on boiling; cool under a tap, and test for proteids, *e.g.* with acetic acid and potassium ferrocyanide (cp. Less. III. § 4).

b. Boil, cool under a tap, and add a little HCl $\cdot 2$ p.c.; the precipitate suspended in the water has been coagulated by boiling and is no longer soluble in dilute acids.

4. **Alkali Albumin.** Treat a little washed muscle as in § 3 but with NaHO $\cdot 1$ p.c. instead of with HCl . On neutralizing a precipitate of **alkali albumin** will be obtained like that of acid albumin; if too much acid is added the precipitate will be converted into acid albumin and re-dissolved. Take in separate test-tubes a little of the precipitate suspended in water.

a. Add a little NaHO $\cdot 1$ p.c., the precipitate at once dissolves and is not precipitated by boiling; with the proteid tests a reaction is obtained.

b. Boil, cool under a tap, and add a little NaHO $\cdot 1$ p.c., the precipitate suspended in water has been coagulated by boiling and is no longer soluble in dilute alkalis.

c. Add a little NaHO $\cdot 1$ p.c. and a little sodium phosphate, with a drop of litmus solution; add drop by drop dilute HCl : on neutralizing no precipitate takes place, add a little more acid a precipitate is obtained, if still more is added the precipitate is re-dissolved.

It will have been seen that proteids in solution (except peptone) on warming with dilute acids or alkalis give rise to acid albumin or alkali albumin respectively; these substances do not coagulate on boiling and so differ from albumin and globulin, and are insoluble in water and so differ from peptone.

5. Test the following properties of **gelatin**, using commercial gelatin.

a. Break up the gelatin into small pieces, add cold water, the gelatin does not dissolve; heat, the gelatin passes into solution.

b. Cool the tube; the solution sets into a jelly provided at least 1% of gelatin is present. Long continued boiling with water lessens the power of gelatinising.

c. To a solution of gelatin add sodic hydrate and a drop or two of copper sulphate; a violet colour is obtained. (Resembling proteids.)

d. Apply the xanthoproteic test; a faint reaction only is obtained.

e. Add acetic acid; gelatin is not precipitated. (Distinction from mucin.)

f. Add acetic acid and potassium ferrocyanide; gelatin is not precipitated. (Distinction from proteids.)

g. Add Millon's reagent; slight red colour on boiling. Pure gelatin does not give Millon's reaction.

h. Add a few drops of tannic acid; gelatin is precipitated.

i. Add a few drops of lead acetate; gelatin is not precipitated. (Distinction from proteids.)

6. *Preparation of Mucin.* Procure the submaxillary gland of the ox. Mince it and grind it with sand, adding gradually .1 p. c. potassium hydrate solution, shake from time to time. In half-an-hour to an hour strain through linen and filter through coarse filter paper. One litre of the alkaline solution to 50 grams of moist gland will give a strong solution of mucin.

To mucin apply the following tests.

a. Add to a solution of mucin acetic acid drop by drop, a stringy precipitate of mucin occurs which is not soluble in excess of the acid. Remove the precipitate on a glass rod and wash it with water, it does not dissolve. Place it in a test-tube and add .1 p.c. Na_2CO_3 , the mucin swells up and slowly dissolves.

b. Its solution does not coagulate on heating.

c. With sodic hydrate and copper sulphate it gives a violet colour.

d. It gives Millon's reaction and the xanthoproteic reaction.

e. It is not precipitated by potassium ferrocyanide, but if the solution is strong it may become viscid. (Distinction from proteids, except peptone.)

f. It is not precipitated by tannic acid. (Distinction from gelatin.)

g. It is precipitated by lead acetate. (Distinction from gelatin.)

h. On boiling with a few drops of 25% H_2SO_4

mucin is decomposed and a reducing sugar formed. Apply Trommer's test (cp. p. 180).

Egg-white.

7. Crack the shell of an egg into two equal parts and allow the white to flow away from the yolk.

Test the reaction of the egg-white. It is alkaline.

8. Stir up the egg-white with a whisk, thus breaking up the membranes which hold it and strain through muslin. Add four times the volume of distilled water and mix thoroughly. A precipitate is formed of a mucin-like substance (ovomucin). Filter this off and apply to it the tests of mucin (see § 6).

9. *a.* To the filtrate add acetic acid drop by drop until the fluid is just acid, a precipitate occurs consisting of globulin (with any remaining ovomucin).

b. Saturate another portion of the filtrate with MgSO_4 . A small precipitate consisting chiefly of globulin occurs.

10. The filtrates from § 9, *a*, and § 9, *b*, contain egg albumin. Apply to them the general tests for proteids.

11. Heat, the solution coagulates.

12. Take the filtrate from § 9, *a*, or neglect the small globulin precipitate due to the addition of acetic acid, and proceed to determine the coagulating point of egg albumin as in Less. III. § 5, *b*. Coagulation begins at about 60°C . but is not complete until 74°C .

13. Take the filtrate from § 9, *b*, and saturate it with Na_2SO_4 so that the solution is saturated with both MgSO_4 and Na_2SO_4 , a bulky precipitate of egg albumin occurs.

14. A small quantity of a mucoid body (distinct from ovomucin) remains in solution after globulin and albumin have been coagulated by heat and may be precipitated from the filtrate by alcohol. It comes down with the proteids on double saturation with Na_2SO_4 and MgSO_4 .

LESSON XI.

PHYSIOLOGY OF MUSCLE AND NERVE. I.

A. APPARATUS FOR STIMULATION.

1. Unless otherwise mentioned one Daniell's cell will be sufficient to use as a **battery**. Ordinary electrodes consist of platinum wires mounted in ebonite and exposed at their ends on one side only, or simply of wires projecting about two centimetres from a stout guttapercha covering.

2. *a.* A key may be used either as a short-circuit or as an in-circuit key. With covered copper wires connect one side of a key with one pole of a Daniell's cell and with one electrode, and the other side with the other pole and other electrode; it is then arranged as a **short-circuit key**. When the key is open the current passes into the electrodes; place the electrodes on the tongue, and note the sensation on opening and closing the key.

b. Connect one side of a key with one pole of a Daniell's cell, the other side of the key with one electrode, and the remaining electrode to the other pole

of the Daniell's cell; it is then arranged as an **in-circuit key**. When the key is shut the current passes through the electrodes.

The **arrangement** *a* or *b* is spoken of as that for a **constant** or **voltaic current**.

c. The keys used are of two types, one, as the du Bois-Reymond key (Fig. 3), in which complete contact is only gradually though rapidly made; and the other, as in the Morse key, in which the whole contact is made practically at the same moment.

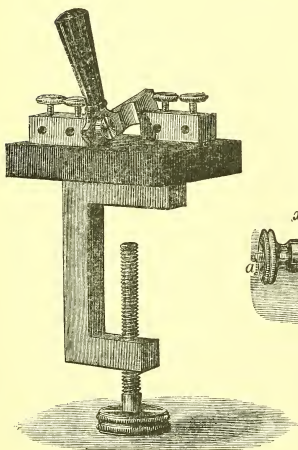


Fig. 3.

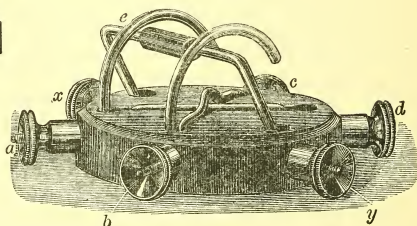


Fig. 4.

d. **Morse Key** (Fig. 5). The following diagram (Fig. 6) shows the way in which the wires of the key are (usually) arranged.

The key is arranged as a short-circuiting key by connecting the battery wires to *a* and *b* (or the wires of the secondary coil),

and the derived circuit (the primary coil, or electrodes) with a and c .

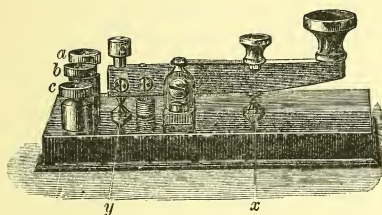


Fig. 5.

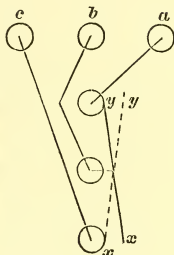


Fig. 6.

The key is arranged as an in-circuit key, by connecting with b and c ; thus if put in the primary circuit of an induction machine the wires from the primary circuit run to b and to one pole of the battery, the other pole of the battery is connected with c . Putting down the key makes the current.

e. A *commutator* for changing the direction of a current (cp. Fig. 4) consists of a disc of ebonite carrying six mercury cups to each of which a binding-screw leads. Into the mercury cups to which the screws x and y , called the central screws, lead, lie the ends of a copper bar e , the two halves of which are insulated from each other by a central mass of ebonite, each half carries a pair of arms, and the bar can be turned so that the arms dip into the cups connected with the screws a and b or into those connected with c and d . These cups are connected diagonally, a to d and b to c , by cross wires not touching each other. The battery is connected to x and y , and if the bar e makes contact with a and b , x is connected to a and y to b ; if it makes contact with c and d , x is connected to b and y to a by means of the cross wires. A current in wires connected with a and b can therefore be changed in direction.

3. **Induction machine.** The end of this is shown in Fig. 7.

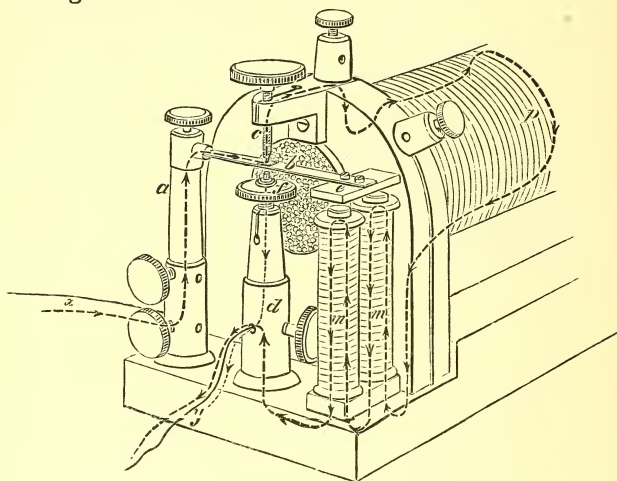


Fig. 7.

a. Connect the poles of a Daniell's cell with the screws at the top of the machine interposing a key. An in-circuit key should be used; it is better, though not necessary for the experiments to be performed in this Lesson, to use a key of the 2nd type mentioned in § 2, *c*. Connect the electrodes with the screws of the secondary coil, interposing a short-circuit key. Put the index of the secondary coil at 10 cm., open the circuit of the secondary coil, place the electrodes on the tongue. Note the sharp shock caused by the make and break of the primary circuit. Move the secondary coil away from the primary, and determine the maximum distance at which the make and the break shock can be

felt. The make shock will probably cease to be felt before the break shock. The **arrangement** is spoken of as that for **single induction shocks** or for induction shocks of slow rhythm.

b. The circuit being open, change the battery wires, from the screws at the top of the machine to those at the base of the two pillars. Screw down *f* (cp. Fig. 7) out of range of the hammer *b*, screw down *c* so that it just touches *b*. Leave the connections of the secondary coil and electrodes as before. Close the primary circuit (if necessary flick the hammer *e*), the hammer will be set in rapid oscillations, each downward movement of the hammer breaking the primary circuit, each upward movement making it. Put the index of the secondary coil at 35 cm., place the electrodes on the tongue, and gradually push the secondary towards the primary coil; note the position at which the shocks are first felt. Note also the position at which the shocks can not be borne comfortably. The **arrangement** is spoken of as that for **tetanizing shocks** or for induction shocks of rapid rhythm. The method in which the primary current is made and broken will be easily understood from the figure. When the current is passing in the primary coil, *b* is in contact with *c*, but since the current also passes round the coil *m* the soft iron core of this coil becomes magnetized, and consequently attracts the plate *e*; as this goes down the contact of *b* and *c* is broken, hence the current ceases to pass in the primary coil, the core of *m* is no longer magnetized, the plate *e* flies up, *b* comes again in contact with *c* and the cycle of events starts again.

c. *Arrangement for equalizing the make and break shocks.* Connect, by a short stout wire, the upper of the two screws on the pillar *a* with the nearer of the two top screws of the primary coil. Raise the screw *c* out of range of the hammer *b*, and raise the screw *f* until it just touches *b*. Close the primary circuit, flick the hammer, and if the screw *f* is properly adjusted the hammer will continue in oscillation. Each upward movement of the hammer makes the primary circuit, and each downward movement of the hammer making contact with *f* breaks the primary circuit, the current passing from the pillar *a* to the pillar *d*, except for a small portion of the current which passes by the side wire connecting *a* with the primary coil. Since the current is not completely broken, the shock induced at the break is less than before, and by adjusting the resistance of the side wire may be made equal to the shock induced at the make.

B. DIRECT OBSERVATIONS.

1. **Constant Current.** Cut off the head of a pithed frog, and squeeze the body upwards to remove most of the blood. (This is done to avoid bleeding, if vessels are cut in dissecting the nerves.) Make a longitudinal incision through the skin of the back of the thigh. With fine-pointed scissors cut through the connective tissue between the large semi-membranosus muscle and the small biceps: the former lies on the inner side of the thigh, the latter is seen along the outer and lower border of the semi-membranosus. The sciatic nerve and the femoral artery will come into view: isolate the nerve by tearing away with a "seeker" the connective tissue around it; if the tissue is resistant it is better to lift up the nerve with a seeker and to cut through the connective tissue with fine scissors. Be careful not to pinch the nerve with the forceps, nor to put more strain upon it than is absolutely necessary.

Place a piece of thin sheet india-rubber, about 1.5 cm. wide under the nerve; arrange for a constant current (A. § 2, *b*), close the short-circuit key, place the points of the electrode under the nerve. Open the key so as to throw the current into the nerve; a **single contraction** or **twitch** of the leg is obtained, the **make contraction**; leave the key open for 5 to 10 seconds, note that there is no further movement of the leg, *i.e.* during the passage of the current without variation of intensity the nerve is not stimulated. (In certain circumstances, stimuli may be set up during the passage of the current.) Shut the key; a single contraction of the leg is obtained, the **break contraction**.

2. Single and slowly repeated induction shocks. Arrange the induction machine for single induction shocks (A. § 3, *a*), with an in-circuit key in the primary circuit, place the index of the secondary coil at 10 cm., open the key of the secondary circuit.

Shut the primary key (*i.e.* make the primary current); there is a single contraction or twitch of the leg, the **make induced twitch**. Open the primary key (*i.e.* break the primary current); there is a single contraction or twitch of the leg, the **break induced twitch**. Open and shut the primary key at intervals of a second for 10 seconds, each make and break of the primary current causes a twitch of the leg.

3. Rapidly repeated induction shocks. Arrange the induction machine for tetanizing currents (A. § 3, *b*). Close the key of the secondary circuit. Make the primary circuit. When the hammer is steadily oscillating open the secondary key; the leg is immedi-

ately thrust out straight and kept rigid in **tetanus**. In a few seconds, break the primary current, the limb at once becomes flaccid. If the stimulus is kept on long, or too often repeated, the muscle will be exhausted; in that case take the nerve and muscle of the opposite side for §§ 4 and 5.

4. **Chemical stimulation.** Cut through the tissue above the sciatic nerve up to a quarter of an inch above the end of the urostyle, and there cut it across, with scissors and seeker isolate the nerve up to the cut. Let the extreme end of the nerve dip into a saturated solution of sodium chloride; watch the leg.

In a variable time twitchings of the toes will be seen, and after a while these will increase in vigour and will extend over the limb until it becomes almost as rigid as in § 3.

5. **Mechanical stimulation.** Cut off the part of the nerve which has been dipping in the salt solution; the chemical stimulus being thus removed, the leg will become flaccid again.

Pinch the remaining nerve sharply with a pair of forceps several times. At each pinch the muscles of the leg will contract. The pinching kills or injures the part of the nerve pinched; hence it is necessary to begin at the cut end, and work down towards the muscle.

6. **Reaction of living and dead muscle.** Have ready a piece of faintly blue, and a piece of neutral litmus paper.

a. Cut out one of the thigh muscles of the opposite

side; cut it across with a clean scalpel, press one surface on the faintly blue, the other on the neutral litmus paper. Both will indicate an **alkaline reaction**.

b. Stimulate the sciatic (peripherally of the part pinched in § 5) with tetanizing currents, pushing the secondary coil over the primary so that the induction shocks are strong to the tongue. Continue the stimulus as long as any effect can be obtained. Then cut across the gastrocnemius muscle, and test its reaction as in *a*. It will be found to be **faintly acid**.

c. Take the gastrocnemius of the opposite side; place it for five minutes in normal salt solution at 45° C. or at a higher temperature. Observe that it becomes contracted and opaque, *i.e.* **rigor mortis** sets in. Test its reaction, it will be **distinctly acid**.

C. OBSERVATIONS BY THE GRAPHIC METHOD.

1. **Tracing of a single muscular contraction.**

Tracings are taken on smoked paper covering a revolving drum, the rate of revolution of which can be varied. First arrange the apparatus for single induction shocks as in A. § 3, *a*, connecting through the screws *s*, *s'* of the moist chamber (Fig. 8) to the electrodes *b*.

Take a pithed frog and cut through the skin, and the skin only, all round the animal at the middle of the trunk, turn the edge of the skin downward, and seizing it with a cloth, tear the skin from the lower half of the trunk and then completely from one leg. Place the frog on its belly on a glass or porcelain plate. Expose the sciatic nerve in the thigh as in B. § 1, and

trace it up to the level of the tip of the urostyle. Lift up the tip of the urostyle with forceps, and with scissors cut through the muscles passing from the urostyle to the pelvic on each side, keeping the scissors close to the urostyle. Free the urostyle in this way right up to its attachment to the end of the vertebral column and then cut it off. The nerves which unite to form the sciatic on each side will be partially brought into view. Place one blade of a stout pair of scissors between the nerves of either side and split the end of the vertebral column in the middle line for about 1.5 cm. With forceps seize the split half on the side selected, turn it outwards, and cut it across transversely 1 cm. from the lower end. Issuing from the piece of vertebral column thus isolated will be seen the 7th, 8th, 9th, and 10th nerves forming the sciatic plexus. Raise the piece of bone, and without stretching the nerves cut through with sharp scissors the muscular and other tissue attached to it, and to the nerves, freeing the nerves as far as the sciatic trunk. In a similar way, cautiously cut through the tissue surrounding the sciatic and so isolate the nerve down to the knee, cutting through its branches on the way. The nerve must not be touched by the forceps; it must be raised only by means of the piece of bone attached to it, and it must not be stretched.

Turn the nerve downwards and let it lie on the muscles below the knee; cut away the muscles attached to the lower half of the femur, taking care not to injure the nerve, and with stout scissors cut through the femur about 1 cm. from its lower end. Divide the lower (Achilles) tendon of the gastrocnemius close to its

attachment at the foot and separate the muscle from below upwards as far as its attachment at the knee, then cut away the tibia and fibula at the knee. The **muscle-nerve preparation** so obtained consists of the gastrocnemius muscle attached to the lower end of the femur, and the sciatic nerve with a piece of the vertebral column attached to it. Pass a hook through the tendon and fix the clamp (*a*, Fig. 8) of the moist chamber to

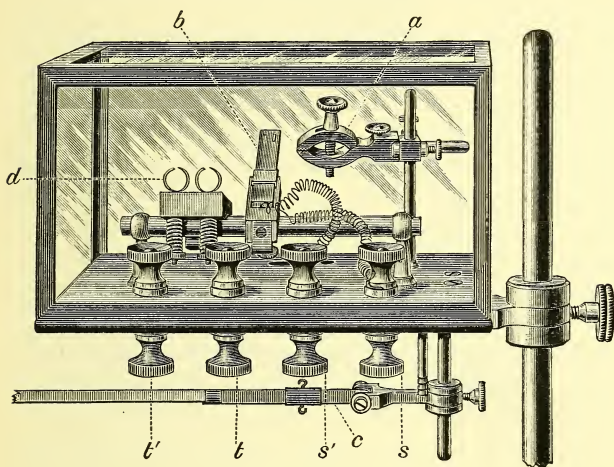


Fig. 8.

the piece of the femur; take up the preparation by the clamp and the fragment of vertebral column; arrange it in the moist chamber so that the muscle is vertically over the hole in the chamber and the nerve lies on the platinum wires of the electrodes *b*, the fragment of

vertebral column being supported on the covered part of the electrodes. Put a piece of blotting-paper well wetted with normal salt solution in the chamber and put on the lid. It is well to moisten the nerve from time to time with a camel's hair brush, wet with salt solution. Attach the hook in the tendon to the lever *c*, load the lever with 10 grams. See that the lever is horizontal and in the same vertical plane as the muscle, and that the hook lies free in the hole in the floor of the chamber.

Bring the lever to mark on the revolving cylinder, adjusting it so that it presses very lightly on the smoked paper. Place the index of the secondary coil at 10. Let the drum revolve at its maximum speed so that the lever may trace a base line, open the key in the secondary circuit, then open the key in the primary circuit, thus sending in a break induced shock. A **tracing of the contraction** will be obtained. Stop the drum and examine the curve. The curve rises rapidly but steadily to a maximum, and almost immediately falls similarly but rather less rapidly, and later near the base line distinctly slowly. In the same way take a tracing of the contraction caused by a make induced shock, with the key in the secondary circuit open, closing the key in the primary circuit. A similar curve is obtained.

Set the cylinder rotating at slower speeds and take further tracings, note the varying form of curve obtained.

2. Latent period. Arrange first the apparatus. A drum is used having a knock-down key : see Fig. 9.

As the drum revolves, a horizontal projecting rod *a* knocks down the vertical rod *b* and so disconnects

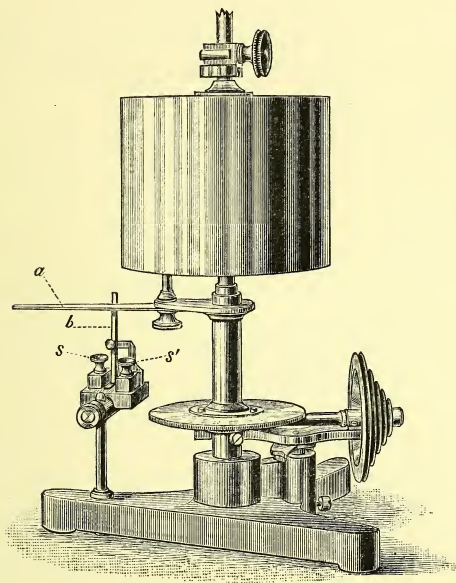


Fig. 9.

the two binding screws *s* and *s'*. Arrange this key as an in-circuit key (A. § 2, *b*), in the circuit of a Daniell's cell and primary coil. Connect the secondary coil with the electrodes of the moist chamber, introducing a short-circuit key. Shut the key of the secondary coil.

Make a muscle-nerve preparation (C. § 1), and arrange it in the moist chamber. Load the lever with 10 grams.

Bring the lever to mark on the revolving cylinder, adjusting it so that it presses very lightly on the smoked paper. Place the index of the secondary coil at 10. Let the drum revolve once so that the lever may trace a base line; stop the drum so that the projecting rod is just beyond the primary key (knock-down key).

Close the primary key, open the secondary key, start the drum at its maximum speed. Stop the drum when it has knocked down the primary key (thus sending a break induced shock into the nerve). Close the secondary key.

The tracing is now obtained; mark the moment of stimulation on the curve, thus. Close the primary key, open the secondary key, turn the drum round by hand until the projecting rod is near the primary key; then very cautiously move the drum till it just touches the primary key and breaks circuit; the muscle will contract and mark on the drum the moment of stimulation. Close the secondary key, and note in the curve

The contraction begins a short time after the moment of stimulation, the time is the **latent period**.

3. *Time measurement of the parts of the curve.* Connect in one circuit two Daniell's cells, a key (in-circuit), a tuning-fork vibrating 100 times a second, and the bobbin of a time-marker, as in the following diagram.

The current flows from the battery by the wire *f*, through the tuning-fork, down the pin connected with the lower prong to the mercury cup *Hg* and so to the binding screw *e*. Here the current divides into two circuits, one passes through the coil *d* lying between the prongs, and the other passes by the wires *g* and *b* through the coils of a time-marker, and both circuits are brought

to the binding screw *a*, from which the current passes to the battery.

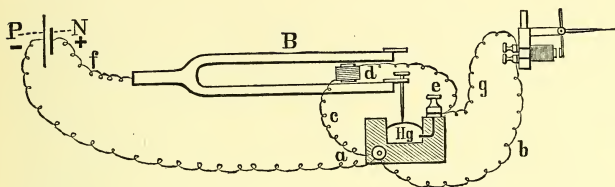


Fig. 10.

The time-marker is shown in the following diagram (a somewhat different form is shown in the preceding figure).

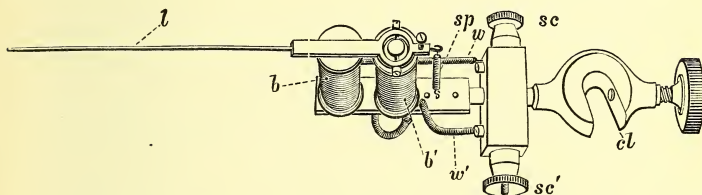


Fig. 11.

The two ends (*w*, *w'*) of the wire wound round the bobbins (*b*, *b'*) are connected with the binding screws (*sc*, *sc'*) to which the current is conducted. When the circuit is made the current passes round the bobbins, the soft core of the bobbins becomes magnetized and the lever (*l*) is drawn down; when the circuit is broken the lever is drawn away from the bobbins by the spring (*sp*). At the end of the lever is fixed a stiff bristle (to write on smoked paper) or a small bent glass tube filled with magenta dissolved in dilute glycerine (to write on glazed paper).

See that the surface of the mercury is quite clean, put on it a few drops of 95 % alcohol, adjust the pin so that it just touches the mercury, flick the tuning-fork, it will continue to vibrate

making and breaking the current at the mercury contact. Each time the current is made the core of the coil *d* of the tuning-fork becomes magnetic, the prongs are drawn together and the current broken, the core then ceases to be magnetic and the spring of the tuning-fork makes the current again. When the lever of the time-marker is oscillating steadily in unison with the tuning-fork (if it does not adjust the strength of the spring of the time-marker), set the drum running at the same rate as it was when the tracing of the single muscular contraction was taken, and bring the point of the lever to write on the drum, immediately below the tracing, while the drum revolves round once. An undulating line is traced, each undulation represents $\frac{1}{100}$ of a second.

Unhook the muscle from the writing lever, letting the lever rest on the finger, adjust its point accurately to the commencement of the contraction, then let it fall and so trace a line downwards to the time tracing. Shift the lever along the curve and drop lines in the same way from the summit of the contraction, the end of the relaxation, and from the line in front of the contraction indicating the latent period. Read the duration of the latent period, the contraction and the relaxation in $\frac{1}{100}$ ths of a second.

4. Minimal and maximal contractions. Disconnect the knock-down key, and substitute an in-circuit key in the primary circuit. Extend the scale of the induction machine to its full length by opening out the half of the scale which lies underneath the machine. Place the secondary coil at 40, open the key in the secondary circuit, then open the key in the primary circuit and so stimulate the nerve with a single break induction shock. Record the height of the contraction if any on a stationary drum. If no contraction results push the secondary coil nearer and nearer to the primary, shifting it half a centimetre at the time, and stimulating the nerve again after each change. In this way find

the position of the secondary coil which just gives a contraction—the **minimal contraction**. Now go on pushing up the secondary coil half a centimetre at the time and record the height of each contraction produced, moving on the drum by hand about one centimetre after each contraction. After each contraction close the secondary key before the primary is closed in order to short-circuit the make induction shock and then open the secondary key before the primary is opened again. Observe that the contractions gradually increase up to a certain point—the **maximal contraction**—and then remain constant although the stimulus is further increased.

Shift the secondary coil back to a position which will give a submaximal contraction on the break of the primary circuit, record the contraction and move on the drum; then leaving the secondary key open, close the primary key and record the contraction due to the make of the primary circuit. This is less than the break contraction, since the induction shock caused by making the current is less than that caused by breaking it. If no contraction is obtained on the make, shift the coil nearer and stimulate by the break and make again. No difference between the make and break contractions will be seen if the stimuli are strong enough to give rise to maximal contractions.

5. Fusion of single contractions to form tetanus. Arrange for single induction shocks, introducing into the primary circuit, in place of a key, an oscillating rod and mercury cup. This is a thin band of steel about 35 cm. long and 2 cm. wide; at one end

at right angles to the band is fixed a pointer 2 to 3 cm. long. The band can be fixed at any part of its length by a wide brass clamp and connected with the clamp is a binding screw. A cup containing mercury is placed underneath the pointer and the clamp is arranged on the stand at such a height that when the band descends in oscillation the pointer dips into the mercury. Shift the oscillating rod in the clamp supporting it, so as to allow its full length to oscillate, and adjust its height so that the pointer just touches the mercury when at rest. Open the key in the secondary circuit, and by moving the oscillating rod in and out of the mercury by hand and shifting the distance of the secondary coil from the primary determine the position of the coil at which contractions are only obtained at break. If such a position cannot be clearly obtained work with a strength of stimulus which is just maximal for make and break so that the height of the contractions is the same. Set the drum running at a rather slow velocity (about 6 cm. per sec.), put the rod into oscillation, then open the key in the secondary circuit for rather more than one second and close it again. Stop the drum, shorten the rod in its clamp by about 4 centimetres, adjust it to the mercury level, and take another tracing of about the same duration. In this way take a series of tracings 6 to 8 in all, with the rod shorter and shorter, shortening it less and less each time. Observe the gradual fusion of a series of single contraction curves into the curve of tetanus.

Finally remove the oscillating rod and mercury cup and connect the battery wires through an in-circuit key to the lower screws of the primary coil (cp. A. 3 b)

and take a tracing, stimulating with the automatically vibrating hammer of the induction coil. A complete tetanus is obtained.

6. Connect in one circuit a Daniell's cell, an in-circuit key, a beating metronome, and a time-marker.

The metronome consists of an oscillating pendulum which (at each oscillation) makes and breaks an electric current lead to two mercury cups. The period of oscillation can be changed by shifting a weight along the pendulum so as to give oscillations at rates varying from 40 to 200 per minute.

Arrange the metronome to beat once a second, and by means of the time-marker record periods of one second duration on the running drum.

Instead of the beating metronome a clock may be used to make and break the circuit, or a chronograph, *i.e.* a small clock with a recording lever, may be brought to bear directly on the running drum.

By means of compasses measure the length on the drum of a period of one second, and count the number of oscillations per second of the muscle in the tracing which just fails to be a complete tetanus.

7. Fatigue of muscle. Arrange the apparatus as for recording the latent period (C. § 2). Connect a second cell to the lower screws of the primary coil as for a tetanizing current, introducing an (open) in-circuit key. Load the muscle with 30 grammes. With the coil at 15 take a tracing of a single muscular contraction and mark the latent period. With the knock-down key open, and the drum at rest, so that the lever will not mark over the tracing just taken, close the tetanizing circuit for 10 seconds, throwing the muscle into tetanus.

Then without shifting the position of the lever, take

another tracing of a single contraction. This second single contraction will probably be recorded immediately above the first, since the relaxation of the muscle after the tetanus may not be quite complete. Then tetanise the muscle for 10 seconds again, and then record another single contraction. Proceed in this way until 8 or 10 contractions have been recorded. Record the latent period of the last of the series. Note that the relaxation of the muscle after each tetanus becomes less and less complete, and the single contractions are recorded immediately over one another. Observe that the second or third contraction is (usually) a little higher than the first, and then the contractions become lower and lower, rising more and more slowly and falling more and more slowly, the prolongation of the relaxation being more marked than that of the contraction, and the latent period is longer.

LESSON XII.

PHYSIOLOGY OF MUSCLE AND NERVE. II.

1. **Extensibility of muscle.** Place a muscle-nerve preparation in the moist chamber and attach the muscle to the lever. Bring the point of the lever to write on the drum, and turning the drum by hand trace a base line with the muscle unloaded (except with the weight of the lever). With a pair of compasses mark off on this a number of points $\cdot 5$ cm. from one another. Put on a load of 20 grammes, the muscle stretches, and the lever falls, recording a nearly vertical line. Move on the drum $\cdot 5$ cm. by hand, and put on another 20 grammes, the lever again falls but less than before. In this way record the magnified extension of the muscle for each successive 20 grammes weight until six or eight have been added, moving on the drum $\cdot 5$ cm. after each addition. The extension becomes less and less, and a curved line is formed if the lower extremities of the lines traced by the fall of the lever are drawn through. When the muscle-nerve preparation is no longer wanted for subsequent experiments remove it; substitute for it a strip of sheet india-rubber, and

test the extensibility of the rubber in exactly the same way. Observe that each weight added stretches the rubber an equal amount and the line joining the extremities of the tracings is a straight one.

2. The work done by a contracting muscle.

Set up the apparatus for taking a single muscular contraction (Lesson XI. C, 1). Load the muscle with 20 grammes placed vertically underneath the muscle, and leave the lever horizontal. Determine the position of the secondary coil which gives a maximal contraction on break of the primary, without being unnecessarily strong. When once decided upon keep the strength of stimulus constant. Trace a base line by moving the drum on about one centimetre. Stimulate with a single break shock recording the contraction on the stationary drum. Close the secondary key, move the drum on about one centimetre by hand, add another 20 grammes weight, the muscle stretches, set the lever horizontal, trace the new base line, and stimulate again with a single break shock. Proceed in this way for each additional 20 grammes added until the load is about 200 grammes, or until the muscle fails to raise the weight. Mark opposite each contraction the load the muscle is carrying at the time. Measure with a pair of compasses the height of each contraction and express it in millimetres. (There is a millimetre scale on the induction machine.) Multiply the height of each contraction by the load the muscle was lifting in each case. The figures so obtained give the relative work done by the muscle at each contraction. Observe that the work done increases as the load increases up to a certain

point and then decreases. Measure the length of the lever from the axle to the point, and from the axle to the attachment of the muscle, and calculate the actual work expressed as gramme millimetres done by the muscle when it was doing its maximum work.

Repeat the experiments, but instead of stimulating the muscle with single break shocks throw the muscle into tetanus (Lesson XI. A, § 3, *b*) for not more than one second each time the load is raised.

3. Effect of heat and of cold on muscular contraction. Take a piece of small lead tubing about .5 cm. diameter, and turn it about 5 times round the end of a rod of 1.5 cm. diameter, and so make a coil of tubing about 4 cm. high. Put this in the moist chamber so that the muscle can hang in the coil, and bring the ends of the lead tubing through holes bored in the floor of the chamber. Put two beakers (of about 2 litre capacity), one to contain ice-cold water, the other water at ordinary temperature, at a level higher than that of the moist chamber, and by means of a bent glass tube to dip into one or other of the beakers and a rubber tube connection; arrange for a flow of water through the coil of lead tubing, controlling the flow by a screw clamp on a rubber tube beyond the coil. Arrange apparatus for taking a tracing showing the latent period (Lesson XI. C, § 2). Place the secondary coil at 15 cm. Fix a muscle-nerve preparation within the coil in the chamber, taking care that neither the muscle nor the nerve touches any part of the coil.

Moisten the nerve frequently with normal salt solution. Take a tracing of a single contraction and

mark the latent period. Let ice-cold water run slowly through the coil, after 3 minutes take another tracing superimposed on the first. Wait 3 minutes more, then take a third tracing. Note cold increases the latent period, prolongs the whole period of contraction, the rise of the lever and the fall, and there is some contraction-remainder. The lift is usually diminished or unaltered.

Shift the bent glass tube to the other beaker and run through the coil water at ordinary temperature (10° — 15° C.), after 3 minutes take another tracing immediately above or below the series already recorded and mark the latent period. This tracing will be more or less of the ordinary form. Raise the temperature of the water to 25° , run it through slowly for 3 minutes, then take a tracing superimposed on the last. Note diminished latent period, whole period of contraction shorter, sharp rise and sharp fall of the lever; the lift will probably be the same. Raise the temperature to 35° , run the water through for 3 min., and take a third tracing superimposed on the last two. Note, the lift is now increased, but the other features of the tracing are much the same as in the last. Raise the temperature to 45° , run the water for 3 minutes, take a tracing, note the lift is now diminished, if it is not, wait a further period of 3 min., and stimulate again, the other features of the tracing are much the same. Mark each tracing to indicate the temperature of the water at the time; that of the muscle is of course less. Shift the position of the lever on the drum and run water at 55° through for 3 min. Note the effect of super-heating—lift may be much increased, sharp rise, long-sustained

irregular contraction, sharp fall. Spontaneous twitching of the muscle will occur as rigor comes on. Let the water continue running and at intervals take 2 or 3 more tracings; note, the lift becomes smaller and the contraction shorter until in 8 to 10 min. no contraction is obtained.

4. **Action of veratrin on muscular contraction.**

Take a frog with the brain only destroyed. Inject 3 or 4 drops of 1 p.c. veratrin solution under the dorsal skin. After 15 to 20 minutes pinch the lower limbs; note that the reflex movements excited are prolonged, the limbs remaining extended for some seconds. Wait until this condition is well marked, then pith the frog and make a muscle-nerve preparation. Load the muscle with 20 grammes, stimulate with a fairly strong single break induction shock, and take a tracing of the contraction with the drum running at its fastest rate. Note that the rise of the lever does not differ much from the normal, while the fall is greatly prolonged, the lever not reaching the base line until the drum has revolved several times. Run the drum slowly, about 2 cm. a second, and take several tracings in quick succession; note that after a time a secondary rise appears on the curve and the relaxation becomes less and less prolonged, and later the secondary rise disappears and the curve becomes nearly normal. Let the muscle rest for a few minutes, stimulate again, a curve with prolonged relaxation is again obtained.

5. **Action of curare on muscle and nerve.**

Take a frog with the brain only destroyed. Expose the sciatic nerve in the middle of one thigh, *e.g.* the left,

(cp. Lesson XI. B, § 1) taking care not to injure the accompanying blood vessels. Gently raise the nerve and pass a ligature underneath it, tie the ligature tightly round all the other structures of the thigh including the blood vessels. With a syringe or small glass pipette inject two drops of 1 p.c. solution of curare underneath the skin of the back. In 20—30 minutes pinch the left and then the right leg; the left to which the poison has no access will be drawn up, the right will not. If the right is drawn up wait until the reflex is completely abolished. Since, on pinching, the left limb is drawn up, the spinal cord and the sensory and motor fibres of the upper part of the sciatic to which the drug has had access are not, at any rate yet, paralysed. Arrange a coil for stimulating with the interrupted current (Lesson XI. A, § 3, *b*). Pith the frog, and expose the sciatic nerves of both limbs from the vertebral column to the knee, and lay bare both gastrocnemius muscles. Stimulate the left sciatic close to the vertebral column where the drug has had access to the nerve trunk, contraction of the gastrocnemius nevertheless results. Stimulate the right sciatic, no contraction of the gastrocnemius is produced in whatever part of its course the nerve is stimulated. Apply the electrodes directly to the right gastrocnemius, contraction is produced. The drug has therefore in all probability paralysed the terminations of the motor nerve in the muscle. Divide the Achilles tendon of the left gastrocnemius and place the muscle in a watch-glass containing curare solution. In 4 or 5 minutes stimulate the left sciatic, no contraction of the gastrocnemius will result if the drug has sufficiently penetrated the

muscle. Stimulate the muscle directly, contraction results.

6. Unipolar stimulation. Set up the apparatus for stimulating with single induced shocks (Lesson XI. A, § 3, *a*). Expose the sciatic nerve in a pithed frog lying on a cork board, place the electrodes under the nerve, guarding them from touching surrounding tissue by a piece of sheet india-rubber. Determine the position of the secondary coil which just gives a distinct contraction on break of the primary circuit. Disconnect one electrode from the secondary circuit key, remove the wire entirely or let it hang over the edge of the table without touching the table. Leave the secondary key open. Break the primary circuit key, no contraction results. Push up the secondary coil several centimetres, and break the primary circuit again until a position is found when a contraction results. Put the cork board with the frog on a dry glass plate, and stimulate again; no contraction results. (If the induced currents are very strong a contraction may still be produced.) Now touch the frog with the finger and while touching it stimulate again; a contraction results. When the frog is not insulated or is touched with the finger the induced currents pass by one electrode through the frog to earth.

Repeat the experiments with tetanic stimulation, the effects are more obvious.

LESSON XIII.

PHYSIOLOGY OF MUSCLE AND NERVE. III.

1. *Rate of transmission of a nervous impulse.* A drum travelling at a rate of at least 100 cm. a sec. is necessary or a rapidly moving pendulum myograph may be used. This consists of a pendulum one metre long, carrying a sheet of smoked glass on which the tracing is taken when the pendulum is moving at its maximum velocity.

Introduce a knock-down key into the primary circuit, to be opened by the revolving drum or the swinging pendulum (Less. XI. C, § 2). Connect the secondary circuit to the central screws of a commutator from which the cross wires have been removed, and lead off from each pair of lateral screws of the commutator to a pair of electrodes in the moist chamber. Arrange a muscle-nerve preparation in the chamber with the nerve lying on both pairs of electrodes placed as far apart as possible, one near the muscle and the other near the distal part of the nerve. Arrange the commutator so as to stimulate the distal end of the nerve, take a single contraction and indicate the latent period. Turn over the bar of the commutator, and stimulating the nerve near the muscle take another tracing superimposed on the first. In this the latent period is shorter; the difference indicates the time required for the passage of the nervous impulse along the length of nerve intervening between the electrodes.

Measure this difference of time by means of a vibrating tuning-fork (Lesson XI. C, § 3), measure the length of nerve lying between the electrodes, and calculate the velocity of the nervous impulse.

2. *Stimulation by the voltaic current. (Law of polar stimulation.)* Take a piece of sheet cork about 4 cm. square, thrust the bare ends of two battery wires twice through it parallel to each other about 2 cm. apart and press the wires down into the cork, so that they hardly lie above the level of the cork. Connect the wires through a short-circuit key to a Daniell's cell. Take a frog which has been injected with urari (the right leg of the frog used in Less. XII. § 5 will serve) and tie a thread to the lower tendon of the sartorius (cp. p. 72, § 2), then raising the muscle by the thread cut the connective tissue along its borders, separating the muscle right up to the pelvis and cut it across close to the bone. Lay the sartorius on the wires in the cork so that one electrode is near the upper and the other near the lower end of the muscle. Fix both ends of the muscle to the cork with hedgehog quills. Watch the muscle carefully, open the key, and so throw in the current; note that the contraction of the muscle remains as a persistent tetanus at the negative pole. In 3 or 4 seconds close the key, cutting out the current; note the remaining contraction at the negative pole ceases, and the contraction of the whole muscle on the break often persists for a short time at the positive pole. With a camel's hair brush apply to the muscle a few drops of .2 p.c. veratrin in normal salt solution and stimulate the muscle again. After a few minutes, as the contractions of the muscle become slower and its irritability diminishes, contraction will be observed only at the kathode on making and at the anode on breaking the current. These will be more obvious if the muscle is observed through a lens.

3. *Variation in the strength and direction of voltaic current. (Law of contraction.)*

Make a pair of *non-polarisable electrodes* as follows.

Take two U-shaped glass tubes to fit the clips (*d*, Fig. 8) in the moist chamber, with one limb 4 cm. and the other 3 cm. long, fill the shorter limb with china clay moistened with normal salt solution. Take care that the clay completely fills the limb of the tube and that no air bubbles are included in the clay, this is best done by pushing the clay in by the fingers only. Wipe the outside of the

tube dry and free from clay ; leave a small wedge-shaped mass of clay projecting from the end of the tube. Put the tubes in the clips in the moist chamber. With a pipette put saturated zinc sulphate solution into the longer limb of the tubes, taking care not to put in too much and that none runs down the outside of the tubes, and insert into the zinc sulphate solution small amalgamated zinc rods carrying covered wires, connect these with the inner screws of t, t' (Fig. 8). Put a piece of moist blotting-paper in the chamber and cover at once.

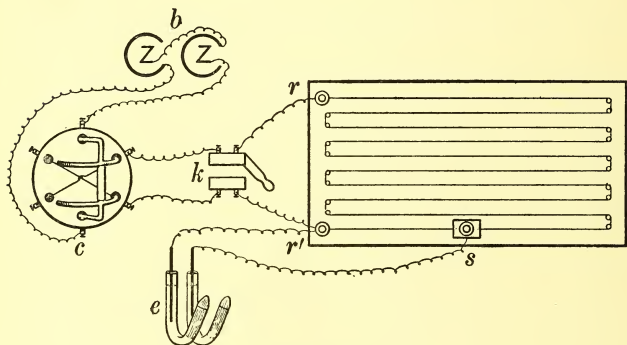


Fig. 12.

Connect two Daniell's cells (b , Fig. 12) to the central screws of a commutator c and lead off from one pair of lateral screws of the commutator to the terminals r, r' of a simple rheochord, introducing a key for short circuiting the current. The rheochord consists of a board carrying two binding screws, to which the ends of a German-silver wire about 3 metres long of 8 to 10 Ohms resistance are connected. The wire passes up and down the board ten times, being carried round ebonite pegs at the top and bottom, and the board is marked transversely by lines, dividing the wires running up and down, into tenths, so that the whole wire is divided into hundredths. A moveable block s carrying a binding screw can be placed on the wire, to make electrical contact with it, at any division. Connect one non-polarisable

electrode to one terminal of the rheochord and the other to the moveable block of the rheochord. Fix a muscle-nerve preparation, the nerve of which has been freed from all adherent connective tissue, in the moist chamber, the nerve lying across the projecting wedge-shaped clay of the non-polarisable electrodes placed about one to two centimetres apart. The portion of spinal column attached to the nerve may be supported on a cork; neither electrode should be in contact with the end of the nerve nor with a cut branch of the nerve.

Arrange the bar of the commutator so that when the current is sent into the nerve it will be descending, *i.e.* the anode furthest from the muscle.

Place the moveable block of the rheochord on the wire of the rheochord close to the terminal to which one of the non-polarisable electrodes is connected. Open the key in the circuit, a minimal current is sent into the nerve. Close the key and shift the moveable block along the wire one division at a time, after each shift open the key and after 3 or 4 seconds close it again. At a certain position of the block contractions will begin to occur when the current is sent in. Record the contractions on a stationary drum, moving the drum a centimetre after each contraction. With weak currents no contractions occur on breaking the circuit. Shift the moveable block further and further along the wire, throwing in the current each time; at a certain position contractions will occur on breaking the current as well as on making it, minimal at first, gradually increasing until the two are equal.

Turn over the bar of the commutator so that the current through the nerve is ascending and take another series of contractions, commencing with the weakest currents. If the nerve is in good condition, a contraction on making will occur with weaker currents when ascending than when descending, since when ascending the kathode, which is the seat of stimulation, is near the upper or more excitable part of the nerve. Except for this, there will be but little difference between the two series.

Remove the rheochord, connect the non-polarisable electrodes through the short-circuiting key to the lateral screws of the

commutator and arrange the commutator so that the current through the nerve will be descending.

Open the key and send in the whole current from the two cells and after 3 or 4 seconds close the key. Observe a large contraction on making and a small contraction, or none, on breaking the circuit through the nerve. Turn over the bar of the commutator so that the current is ascending and stimulate again, a small contraction occurs at the make, a large contraction at the break. Stimulate with still stronger currents, by introducing one or two more cells into the circuit; observe that a strong descending current gives a contraction at make but not at break, while a strong ascending current gives one at break but not at make.

4. *Change in irritability of a nerve produced by a constant current. (Electrotonus.)*

Make a pair of non-polarisable electrodes (§ 3) and place them in the moist chamber.

Connect them with two Daniell's cells, introducing a rheochord, a commutator and a key for short circuiting the current (cp. Fig. 12). A signal may be introduced into the circuit between the key and the rheochord and its writing-point placed immediately underneath the point of the muscle-lever, when the current is sent in, the signal will indicate the period of closure.

Arrange a coil to give single induction shocks to the nerve by the platinum electrodes of the moist chamber.

Lay the nerve of a muscle-nerve preparation on both pairs of electrodes, the platinum electrodes being nearest the muscle and as close as possible to one non-polarisable electrode, the non-polarisable electrodes being separated from one another about two centimetres.

Run the drum at its slowest rate about 2 mm. a sec.

Arrange the commutator so that the non-polarisable electrode nearest the platinum electrodes is the kathode. Separate the coils of the induction machine so that a single break shock produces a minimal contraction. Record three or four contractions, stimulating regularly every two seconds, opening the key with the same rapidity each time. Place the moveable block of the

rheochord on the wire near the terminal to which one of the non-polarisable electrodes is connected and adjust it so that a contraction is produced at the make only of the constant current. Open the key in the constant current circuit. Stimulate with single break induction shocks regularly every two seconds. Larger contractions are now produced (increased irritability). Close the key in the constant current circuit. Go on stimulating regularly six or eight times immediately after the current is cut out. At first no contractions are produced (after-effect of diminished irritability), later minimal contractions are produced as before. Irregularity will probably appear owing to variation in the rate of break of the primary circuit key.

Arrange the commutator so that the anode is nearest the platinum electrodes and send in the constant current. Push up the secondary coil slightly and send in single break induction shocks which give small contractions, *i.e.* sub-maximal contractions. Now stimulate with the single induction shocks, smaller contractions or no contractions result (decreased irritability). Break the constant current circuit. Go on stimulating regularly immediately after the current is cut out. At first maximal contractions are produced (after-effect of increased irritability), later submaximal contractions as before. Irregularity will probably appear for the reason given above.

5. *Change in irritability of a nerve produced by change of temperature.* Take a piece of glass tubing about 3 cm. long and connect its ends to rubber tubing brought through holes bored in the floor of the moist chamber. Support the glass tube on a cork, placing it so that the nerve can lie conveniently across it. Arrange for a flow of cold water (5° C.) and warm water (30° C.) through the tubing (cp. Less. XII. § 3). Connect two Daniell's cells through a commutator, a key (short-circuit) and a rheochord (cp. Fig. 12) to a pair of non-polarisable electrodes placed in the chamber. Lay the nerve of a muscle-nerve preparation across the glass tube and on the electrodes, so that the tube is nearest the muscle and quite close to one non-polarisable electrode (or place the lower electrode on the part of the nerve which lies on the tube). Arrange the commutator so that the cathode is nearest the glass tube. Set the drum running at its slowest rate. Adjust

the moveable block of the rheochord so that minimal contractions are obtained on throwing in the constant current. Take six or eight contractions, stimulating regularly every two seconds. Then run the cold water through the tube, stimulating regularly all the time. The period during which the cold water is running may be indicated by a signal in an independent circuit. The contractions will rapidly increase and soon become maximal. When there is no further increase, stop the flow of cold water and run through water at 30° C., stimulating regularly all the time. Note the contractions rapidly diminish, until, probably, no contraction is produced. Run cold water again and note the gradual increase of the contractions to maximal again. Cold increases and heat diminishes the excitability of nerve to the make of the voltaic current (when the current is closed for at least $\frac{1}{100}$ sec.).

Arrange the strength of the constant current so that a contraction is given at make and at break. Observe the effect of a rise and of a fall of temperature on the make and break contractions with ascending and with descending currents. Evidence will be obtained that the stimulus is set up at the cathode at the make, and at the anode at the break of the current.

6. *Demarcation current of muscle and its negative variation.* Arrange a Thomson's reflecting galvanometer of high resistance (10,000 to 20,000 Ohms) to reflect a beam of light on to a scale. Connect the terminals of the galvanometer to a *shunt*, i.e. a resistance box, with resistances of such relations to the resistance of the galvanometer that $\frac{1}{10}$, $\frac{1}{100}$, or $\frac{1}{1000}$ of a current led to the shunt may, at will, be sent through the galvanometer and $\frac{9}{10}$, $\frac{99}{100}$ or $\frac{999}{1000}$ be short-circuited by the shunt. Set up apparatus for stimulating with tetanizing shocks by platinum electrodes (Lesson XI. A, § 3, b), leading from the secondary coil by long wires to the short-circuiting key, so that the coil may be 3—4 yards from the galvanometer.

Take a small zinc rod such as is used for non-polarisable electrodes (cp. § 3) and a copper battery wire, and dip them into dilute (1 in 20) sulphuric acid in a watch-glass and connect them with the terminals of the shunt, having previously inserted a plug between the terminals, so that all the current is short-circuited from the galvanometer. Insert a plug in the shunt so

that $\frac{1}{1000}$ of the current will pass through the galvanometer, then momentarily remove the plug from between the terminals, note in which direction the spot of light on the scale moves, and which terminal of the shunt you have connected with the zinc rod, *i.e.* made the negative pole. In place of the copper wire and zinc rod, connect to the shunt a pair of non-polarisable electrodes made as in § 3, except that a thick worsted thread, moistened with salt solution and covered with clay, is to be inserted with the clay and left projecting from the tube just enough to support and render slightly moveable the clay which projects from the tube. The electrodes may be held in any convenient clamps standing on a sheet of glass or in the clips of the moist chamber, the whole chamber being placed on glass. Bring the projecting clay of the electrodes into contact and test them to see if they are iso-electric by sending first $\frac{1}{10}$ and then the whole of any current there may be through the galvanometer. If there is much deflection the electrodes are not properly made. Make a fresh pair. If there is but little deflection, note its amount and which electrode is negative. Cover the electrodes with blotting-paper wet with normal salt solution to prevent them getting dry.

Make a muscle-nerve preparation, taking care not to injure the muscle. Fix the muscle horizontally by two clamps, one holding the femur and the other the Achilles tendon, so that it cannot shorten on stimulation. Bring the non-polarisable electrodes into contact with it, so that one touches the muscle at its belly and the other touches it close to the Achilles tendon. Lay the nerve over the platinum electrodes connected with the coil.

Remove the short-circuiting plug of the shunt and send the whole of any current there may be through the galvanometer. A small deflection is usually obtained and the deflection is in the direction which indicates that the tendon region of the muscle is negative to the belly. In reading the deflection make any correction that is necessary, if the electrodes were found to be not iso-electric. Short circuit the current at the shunt, with scissors snip the muscle close to the tendon without cutting it through and bring the electrode in contact with the injured spot. Test the electrical condition again; a large deflection is obtained, the spot of light probably passes off the scale. If it does, send in $\frac{1}{10}$ only of

the current. This is the *demarcation current* or current of injury. Note that the injured part is negative. Probably at first the spot of light will not remain still, but will move slowly towards zero, indicating that the current of injury is diminishing. After a short time this will probably cease and the spot become steady. When it is steady stimulate the nerve with tetanising shocks of moderate strength for 2 or 3 sec. Note that the spot of light moves rapidly towards zero and then comes to rest, indicating a *negative variation* of the demarcation current as long as the stimulation lasts.

7. *Stimulation by the demarcation current and by the current of action.* (*Rheoscopic frog.*) Arrange a coil for single induction shocks. Take a pithed frog, remove the skin from the legs and dissect out both sciatic nerves from the vertebral column to the knee. Suck up with blotting-paper any blood or lymph from the legs and place the frog on a clean dry cork board. With a scalpel make a transverse incision into the extensor muscles of one thigh, *e.g.* the left, just above the tendon. With a glass rod lift up the left sciatic, from which the piece of vertebral column is removed, and let it fall across the thigh muscles so as to come in contact with the surface and the cut end. As the nerve makes contact, a contraction of the muscles supplied by it will occur. The demarcation current of the thigh muscles stimulates the nerve. Divide the Achilles tendon of the right gastrocnemius, fix the tendon by a pin close to the left knee, and, taking care not to injure the right sciatic, fix the right knee with a pin. Raise the left sciatic with a glass rod and place it across the belly of the right gastrocnemius with its cut end lying close to the tendon of the muscle. Stimulate the right sciatic with single induction shocks, each time the right gastrocnemius contracts the left contracts also. The current of action in the muscle stimulates the nerve.

DEMONSTRATIONS.

1. Isometric contraction.
2. Electrotonic currents.
3. Current of action shown by the capillary electrometer.
4. Ergographic records in man.

LESSON XIV.

NERVE FIBRES.

1. **Spinal Nerves.** Cut off $\frac{1}{2}$ to $\frac{3}{4}$ of a centimetre of a small, perfectly fresh nerve (*e.g.* a branch of the sciatic of a frog), and place it on a glass slide *without any fluid*. Fixing one end by pressing on it with the blunt end of a scalpel, pass a needle through the other end in the direction of the nerve fibres, and so spread them out into the shape of a fan; add a drop of normal saline solution, and cover with the cover-slip. Observe (h. p.)

The **medullated nerve fibres** of variable size. Measure the diameter of some of these (Less. II. § 1).

In each fibre the double contour, due to the **medullary sheath**.

The **primitive sheath** or **neurilemma**; this is seen with difficulty except at points where the medullary sheath has been displaced in mounting.

The **nodes**; these will be seen as short but distinct breaks in the medullary sheath.

Drops and fragments of the medullary sheath, extending from the cut ends of the fibres and showing a double contour.

2. Treat a piece of nerve as in § 1, but add a drop of .2 p.c. nitrate of silver instead of salt solution. Note the rapid staining of the cement substance at the nodes. Expose the specimen to light for half-an-hour. Take off the cover-slip. Dip the nerve in distilled water to remove the silver solution. Mount in dilute glycerine, spreading out the fibres of the teased end. Note the small dark crosses formed by the nodal cement substance and the axis cylinder near the nodes. Probably the axis will also be stained at and near the cut ends of the fibres.

3. Take a short piece of nerve, and tease it into small bundles of fibres in a drop of .75 p.c. sodium sulphate. Run off the fluid, keeping the fibres on the slide with a needle. Add another drop of salt solution, and run it off. Repeat this. Then add a drop or two of .2 p.c. nitrate of silver, and expose to light for about half-an-hour, avoiding drying. Wash in distilled water in a watch-glass, and mount in dilute glycerine, so that most of the fibres are parallel to one another. The staining will thus be more localized at and near the nodes than by method, § 2.

4. Tease out a piece of nerve as in § 1. Add chloroform instead of salt solution, adding more chloroform as evaporation goes on.

In the middle of the nerve fibre the transparent *axis cylinder* will be seen running through the swollen medullary sheath.

5. Take another piece of fresh nerve, and place it in a small quantity of osmic acid .5 p.c. for $\frac{1}{4}$ to $\frac{1}{2}$ an hour, covering it up to prevent evaporation. Wash it in water, place it in dilute glycerine for a minute or two. Remove to a slide and tease. Select a small bundle separated from the connective tissue sheath, and put the rest back in glycerine for use if required.

Tease further the small bundle, and arrange the fibres parallel to one another; place a small drop of dilute glycerine on the centre of a cover-slip, and mount.

Select a nerve fibre which is isolated for a considerable part of its length, and observe

The medullary sheath, stained black with osmic acid.

The **nodes**; note that the distance between two successive nodes is greater in large than in small nerve fibres.

The numerous oblique breaks in the medulla, dividing it into short overlapping cylinders.

The **nuclei** of the sheath; there is one to each internode about halfway between the two nodes, it is an inconspicuous transparent elongated body, usually projecting into the medulla.

The nuclei of the sheath may be stained by placing a piece of the nerve, after brief treatment with osmic acid, in picrocarmine or in hæmatoxylin for an hour. In the former preparation, the cells of the fine connective tissue around the nerve fibres will also be well seen.

6. Transverse sections in paraffin of a large nerve stained with picrocarmine (potassium bichromate 2 p.c.). Mount in balsam. Observe

The epineurium surrounding the whole nerve and passing between the nerve bundles; its structure resembles that of areolar tissue (p. 56).

The perineurium surrounding the nerve bundles, consisting of two or more concentric nucleated membranes.

The endoneurium between the nerve fibres, consisting of a small quantity of fine fibrous tissue.

The cut ends of the nerve fibres varying in diameter,

and in each the section of the stained axis cylinder surrounded by a transparent ring indicating the former position of the medullary sheath, which has been dissolved or made transparent in the process of mounting. The primitive sheath as a limiting circle.

7. Cut through the skin of the frog in mid dorsal line. Cut out one of the small dorsal cutaneous nerves issuing from the muscles of the back and running to the skin. Place it in .2 p.c. nitrate of silver for 5 to 10 minutes. Wash in water and expose to light for about $\frac{1}{2}$ an hour. Dehydrate and mount in balsam. Note the layer of epithelioid cells—shown by the staining of their cement substance—covering the nerve. The nerve will be seen taking a somewhat sinuous course inside the epithelioid sheath.

8. **Sympathetic Nerves.** Cut out from the fresh spleen of a large animal (*e.g.* ox) a small piece of one of the large sympathetic nerves running alongside the blood vessels. Remove the connective tissue sheath, and tease out the nerve carefully in normal saline solution. Note

a. The scanty medullated nerve fibres.

b. The **non-medullated** nerve fibres constituting the bulk of the nerves. Add acetic acid to bring out more distinctly the elongated nuclei attached to them at short intervals.

9. Tease out in dilute glycerine a small piece of the cervical sympathetic nerve (osmic acid) of a cat or rabbit.

Note the numerous **small medullated** fibres; some non-medullated fibres may also be seen. Compare the size of the medullated fibres here and in the sciatic nerve (§ 1).

10. **Peripheral Course of Nerves.** In the specimen prepared Less. IX. § 4: observe

a. Under a low power, the nerve running across the lower part of the muscle and sending off fibres or bundles of fibres at intervals and so spreading out over it.

b. Under a high power, that where the lateral bundles (especially the smaller ones) are given off, one or more of the nerve fibres divide into two fibres, the division taking place at a node. Trace a small bundle of nerve fibres, the nodes are very close together. Each nerve fibre apparently ends abruptly over a muscle fibre; in this specimen it can only be traced as far as the blackened medulla extends.

11. Examine the gold chloride preparation (made in Less. VII. § 6) of the cornea. Note

a. The small separate bundles of nerve fibres entering the cornea at its periphery; the medullated fibres on account of their medulla are more deeply stained than the non-medullated fibres.

b. Trace as far as possible the course of one of the nerve bundles; the medulla soon disappears, the fibres, still showing nuclei at intervals, join with the fibres from other bundles to form a coarse plexus; from this proceeds a plexus of smaller bands which have few nuclei; finally from this plexus run very fine varicose non-nucleated nerve fibrils in straight lines across the cornea (these fine fibrils may also be seen forming part of the finer bands of the plexus). The coarse plexus is the primary plexus of the cornea.

12. *Neurokeratin network.* Tease out in clove oil a piece of nerve which has been extracted with alcohol and ether¹. Mount in balsam. Note the distinct network in the region formerly occupied by the outer part of the myelin sheath. The size of the meshes varies in different fibres.

DEMONSTRATIONS.

1. Transverse sections of sciatic, vagus, and cervical sympathetic nerves. (Cat or rabbit; osmic acid.) Note the relative size of the nerve fibres in the three nerves, and the number of non-medullated fibres in the vagus.

2. Transverse section of non-medullated nerve (splenic nerve of large animal; osmic vapour). Note the oval or circular outlines of the fibres; they vary very little in size, being generally 2μ in diameter.

3. Teased degenerating nerve fibres (4 days after section; osmic acid). Note the myelin broken up into masses and globules of various size; in places a considerable stretch of the nerve may show very little alteration.

4. Similar nerve fibres in later stage (20 days after section). The myelin has nearly all disappeared, a few fibres still show here and there rows of fat globules.

¹ Place pieces of nerve in absolute alcohol for a day, boil in the alcohol on a water bath for about 15 minutes and place in ether for a day. Part should be stained with hæmatoxylin, and part with osmic acid. The pieces may be kept in clove oil.

LESSON XV.

PERIPHERAL GANGLIA AND NERVE CELLS.

1. Transverse section of **spinal ganglion**¹. (Osmic acid; paraffin.) Mount in balsam.

a. Note with a low power, the sheath of the ganglion; the numerous nerve cells having between them medullated nerve fibres, comparatively few nerve fibres being present between the more peripheral cells; the transversely cut nerve fibres chiefly grouped at one side of the section. (The fibres of the anterior root may form a separate bundle with a distinct sheath.)

b. Note with a high power

The **nerve cells** of various sizes, the small ones as a rule being stained more deeply than the large ones; the cell substance varying from nearly homogeneous to coarsely granular; the large spherical nucleus, and large nucleolus or nucleoli.

The numerous large **nerve fibres**; some fibres will be seen coiling amongst the cells in the plane of the section.

¹ In §§ 1 to 3, the ganglion of a cat or dog may be taken. Section 1 and section 4 should be mounted on the same slide.

The **sheath of the ganglion** consisting internally of several membranes, and externally of loose connective tissue.

2. Sections of a spinal ganglion (potassium bichromate; cut frozen) are given you in water. Stain a section in hæmatoxylin and mount in balsam.

Note around each nerve cell the numerous **nuclei of the capsule**.

3. Sections of spinal ganglion (mercuric chloride) given in paraffin. Take two cover-slips, fix sections to them, and treat them for staining on the cover-slip (Less. iv. § 13) up to the stage of placing them in 50 p.c. alcohol.

a. Place one slip in Nissl's methylene blue for about 20 minutes (or warm it with the solution in a watch-glass, till the fluid begins to steam). Blot it between folds of blotting-paper to remove excess of fluid; place it before it dries in a mixture of 10 parts aniline oil and 90 parts 95 p.c. alcohol. Gently move it about in this, till the blue colour of the section is rather faint (the proper tint can only be told by trial); then blot again, transfer to absolute alcohol for 30 to 60 seconds. Clear in xylol, mount in balsam, taking care that the balsam is added to the cover-slip before the xylol evaporates as the sections become dry.

Note in the nerve cells the *basophil masses and granules* stained deep blue, they vary in number and size in different cells, and are often absent from the peripheral zone; they are for the most part arranged concentrically around the nucleus.

b. Place the other slip for 1 to 1½ minutes in erythrosin (or eosin), blot, and treat as in *a.* The erythrosin stains the parts of the sections left unstained by methylene blue.

Methylene blue in 75 p.c. alcohol or Löffler's methylene blue may be used instead of Nissl's methylene blue; 95 p.c. alcohol may be used instead of aniline oil and alcohol; but this is apt to produce somewhat diffuse staining.

4. Transverse section of a **sympathetic ganglion** from the sympathetic chain. (Osmic acid; paraffin.) Under both low and high power compare this section with that of the spinal ganglion in § 1. Note

The nerve cells vary in size, but on the whole are not so large as in the spinal ganglia; they are more irregular in shape, and a considerable number of non-medullated fibres lie between them.

The great majority of the medullated fibres are small (2μ to 3μ).

5. Transverse section of lumbar **spinal cord** of ox, calf, or dog. (Potassium bichromate; cut frozen; picrocarmine.)

Note the large **multipolar nerve cells** of the anterior cornu; the processes are given off in all directions, some are cut short, others will be seen to divide in the section into finer and finer branches (protoplasmic processes or **dendrons**); here and there a cell may be seen from which a rather large process is given off which runs outwards through the white substance without branching (axis cylinder process or **axon**).

6. Tease out in formic glycerine a small piece of the spinal ganglion of a skate (ganglion in 30 p.c. alcohol for 3 days, one day in picrocarmine, washed; kept in glycerine).

Note the **bipolar cells**, forming oval to spherical nucleated swellings on the course of a nerve fibre. The connective tissue sheath of the nerve will be seen to run over the cell and form its capsule.

7. *Spiral nerve cells of the auricular septum of the frog.*

Take a pithed frog: expose the heart, cut through the pericardium, lift up the edges and cut it away so far as it is seen. Turn the ventricle forward, cut through its ligament, lift up the heart by the ligament and pass two silk threads under the aortæ. Draw one backwards, and tie, underneath the ventricle, the veins running to the heart.

With scissors make an incision into the bulbus just before it branches, being careful not to cut it through. Squeeze the heart a little to empty it, wipe away the blood with a piece of sponge moistened with normal salt solution. Put a fine pointed cannula provided with a short piece of india-rubber tubing into the bulbus, prop up the tubing so that the cannula will not slip out, tie it in the bulbus. By means of a pipette fill the cannula with salt solution, squeeze the tubing and so force the salt solution into the heart. Remove the fluid from the cannula by means of the pipette, fill with fresh salt solution, and so on till the fluid returning to the cannula from the heart is colourless.

Empty the cannula, fill it with .5 p.c. gold chloride, take a glass rod which fits the tubing, and slowly push it in so that the heart is distended with gold chloride. See that the right auricle is distended for a minute or two; then lift up the heart by means of the cannula and the thread tied round the veins, and cut peripherally of the ligatures. Place the heart in a little gold chloride solution in a watch-glass for half-an-hour. Transfer to water, cut away the bulbus with the cannula, the superfluous tissue round the auricles, and two-thirds of the ventricle. Move about in the water, and place in 25 p.c. formic acid in the dark till next day.

Then wash in water, and under a dissecting lens cut through the projecting wall of one auricle, note the position inside of the thin membrane, the septum auricularum, and cut away from it the auricles and the rest of the ventricle. Mount it in formic glycerine. Note (a) the two main strands of nerve fibres running through it; the medullated fibres in these will be stained more deeply than the non-medullated fibre. (b) The septum itself formed of a plexus of cardiac muscle cells in a connective tissue membrane. (c) The numerous nerve cells attached to

the nerve-strands. The nerve cells are generally pear shaped. The spiral process may be seen coiling round the straight process, but it is not usually distinct.

(The heart may of course be injected from the inferior vena cava; the method described above is recommended because it is perhaps easier to insert a cannula into the bulbus than into the vena cava.)

The heart may be injected with alcohol; in this case inject weak before strong alcohol, and colour the strong alcohol with a little eosin, so that the septum may be more easily seen. Pass scissors down the bulbus into the ventricle and cut; cut off three-fourths of the ventricle, pass scissors from the ventricle into one auricle and cut. The septum can then be readily separated under a dissecting lens, and stained with any reagent desired.

8. *Nerve cells of Auerbach's plexus.* Cut through and reflect the abdominal walls of a recently killed mouse or rat. Cut out about two inches of the small intestine, tie a cannula in one end, and using a syringe wash out the intestine with normal salt solution. Tie the free end. With a pipette fill the cannula with 1 p.c. gold chloride, distend the intestine with this, tie just below the cannula, whilst the intestine is still distended. Place it in a watch-glass in gold chloride solution for half-an-hour. Remove to water; cut open lengthways, and wash well. Place in 25 p.c. formic acid in the dark till next day.

Scrape off the mucous membrane, and mount in formic glycerine two pieces of the muscular coat, one with the outer, the other with the inner surface uppermost. Note the wide meshed plexus of Auerbach between the muscular coats; the meshes are rectangular and groups of close-set small cells lie at the nodal points, their nuclei staining less than the cell substance. The plexus of Meissner may be seen in places in the sub-mucous layer; it is similar to that of Auerbach but the meshes are irregular in shape, the strands and groups of nerve cells smaller.

9. *Ganglia of the sympathetic chain in the frog.* Lay open the abdomen of a recently killed frog, raise the rectum, and

remove the intestine, liver and stomach, cutting through the mesentery just above the kidneys. Lift up the kidneys and cut between them. Lift up each kidney in turn, the peritoneum—separating the abdominal cavity from the lymphatica cisterna magna—will be seen stretching from the lateral edge of the kidney to the body wall. Remove the kidneys with this part of the peritoneum. Sponge up any blood that may be present.

Note on either side of the aorta a longitudinal pigmented strand, the sympathetic chain, and the fine nerves—the rami communicantes—connecting it with the adjacent spinal nerves. With the aid of a lens, the ganglia may be seen as spindle shaped swellings on the sympathetic chain.

Remove the chain on one side; stretch it out on a slide, cover, and add normal salt solution. Examine with a high power. Note the nearly globular, transparent nerve cells; much hidden by the pigment cells. The nerve fibres of the rami are chiefly small medullated fibres.

By treating the sympathetic chain with dilute methylene blue as directed in Less. XVI., a cell will here and there be seen with deep blue spiral fibre and showing the branchings of the spiral fibre (synapses) on the cell.

Teased ganglia, whether they are taken fresh, or after treatment with reagents, do not give satisfactory preparations of nerve cells, as the process or processes are usually broken off short.

DEMONSTRATIONS.

1. Longitudinal section of spinal ganglion, to show the anterior and posterior roots attached to it, the anterior root running past the nerve cells and joining the fibres of the posterior root at or near the lower end of the ganglion. *

2. Section to show basophil granules and masses of spinal ganglion cells (cp. § 3).

3. Section of spinal cord of mammalian embryo or of chick prepared by Golgi-Cajal method (cp. p. 341), showing multipolar cells with a single axon, and many dendrons.

4. Section of spinal ganglion of chick prepared by Golgi-Cajal method, showing unipolar cells with dividing process.

5. Septum auricularum of frog (cp. § 7).

6. Plexus of Auerbach in intestine of mouse (cp. § 8).

LESSON XVI.

ENDINGS OF EFFERENT NERVE FIBRES IN MUSCLE.

1. **The End-Brush.** Take a pithed frog, and expose the sartorius muscle (cp. Less. IX. § 2). Free this at its lower end; raise it by the short tendon and cut through the connective tissue on either side and below it, close to the muscle, being careful not to injure the muscle fibres. Cut through the upper attachment. Place the muscle on a slide, deep side uppermost, add two drops of .05 p.c. methylene blue dissolved in .6 p.c. NaCl. Line a watch-glass with moist blotting-paper, and with it cover the specimen. Leave for 15 to 20 minutes; then drain off the methylene blue. Gently pull the ends with needles to extend the muscle, add one drop of normal salt solution, and examine with low objective and high ocular.

Follow the muscle fibres along, until the small rather opaque nerve entering the muscle is seen; trace the course of the nerve across the muscle fibres. Here and there, one or two nerve fibres will be seen leaving

the nerve, running to the muscle fibres, and ending on them in blue stained nerve-endings.

Selecting a well stained nerve-ending or **end-brush**, note that in nearly all cases the branches are given off at right angles to the stem, and that they are not very close together. Fine nerve fibrils accompanying the blood vessels will also be seen. When a number of endings have been examined, put on a cover-slip, gently press. With a low power, put a typical ending in the middle of the field, and then examine it with a high power.

Soon after the cover-slip is placed on the muscle, the methylene blue begins to be reduced to a colourless compound, and in consequence the nerve fibres become paler till they are no longer seen.

Note. The success of this preparation depends largely upon not injuring the muscle fibres. Where they are injured, they stain deeply; they should have no stain at a time when the nerve-endings are deep blue.

2. *Preservation of nerve-endings stained with methylene blue.* When the nerve-endings are distinct under a low power, the muscle is pinned out with hedgehog quills, and placed in a saturated aqueous solution of picrate of ammonia for 10 to 15 minutes.

It is then transferred, without washing, to the following solution,

Ammonium molybdate	1 gram.
Distilled water	20 c.c.
Hydrochloric acid	1 drop,

and left for $\frac{3}{4}$ to 1 hour. It is finally washed with water, passed through alcohols, and xylol, and mounted in balsam.

3. Make a similar preparation of the sterno-cutaneous muscle of the frog (Less. ix. § 4). The nerve-endings resemble

those of the sartorius, but in general are not so large or so regular.

4. Reflect the skin from the lower jaw. Observe with a dissecting lens the mylo-hyoid muscle on either side, attached to the edge of the jaw, and, in the middle line, to its fellow muscle. Carefully cut this out, and treat in the same manner as the sartorius (§ 1). Note in it the plexus of fine nerve fibres and fibrillæ throughout the muscle, and the small and irregular nerve-endings.

5. *End plates.* Take a piece about an inch and a half long, of a recently pithed snake. Cut through the skin in the mid-ventral line. Cut through on one side the muscle attached to the skin near the mid-ventral line, and pin out the skin. Take the edge of the ribs in forceps, and pull the body gently away from the skin and upwards. A series of small band like muscles will be seen passing from the body to the skin. Pin back the body so that these muscles are stretched, carefully tear away the tissue on either side of the muscles, isolating them up to their connections at either end. Cut out the muscles and treat them with dilute methylene blue, in the manner given in § 1.

Isolate similarly the muscles of the opposite side up to their origin and insertion; arrange the pins so that they are well stretched; brush them with 1 p.c. gold chloride. In about 5 minutes, when the muscles have become stiff, cut quickly the attachments, remove with a glass rod to gold chloride for about 25 minutes. During this interval examine the muscles treated with methylene blue, placing on a slide, and moistening them with normal salt solution. Observe in the end plates, the curved branches of the axis cylinder set closely together, the whole end plate forming an oval or round patch.

The muscles which were treated with gold chloride may now be removed to water and washed thoroughly. Place in 25 p.c. formic acid in the dark till next day. Wash and place in formic glycerine, put under a dissecting lens, and tease off as much connective tissue as possible. Mount in formic glycerine, press

the cover-slip to separate the fibres a little, and examine for end plates, using a lamp for illumination.

6. Nerve-plexus in unstriated muscle. Cut out the œsophagus of the frog used in § 1. Cut it open, scrape gently the mucous surface. Pin it out with hedgehog quills over a hole in a piece of cork, muscular coat uppermost. Cut off the quills close to the membrane. On the muscular coat place a couple of drops of .05 p.c. saline methylene blue. Cover up with a watch-glass lined with wet blotting-paper for 20 to 30 minutes. Then wash off the blue with a drop or two of .6 p.c. salt solution. Place the piece of cork on a slide and examine with low objective and high ocular, and without diaphragm.

Note the very close plexus of fine blue-stained nerve fibrils, generally varicose in the muscular coat.

Put on a cover-slip and examine with a high power.

7. Make a methylene blue preparation of the nerve plexus in the muscular coats of the rectum of the frog, treating it in the manner given in § 6.

DEMONSTRATIONS.

Microscopic specimens showing motor nerve-endings in snake's muscle. (Cp. § 5.)

LESSON XVII.

REFLEX AND AUTOMATIC ACTION.

A. REFLEX ACTION.

1. A frog is given you which has been deprived of its brain but not of its spinal cord (cp. App. p. 376). Place it in the sitting posture. Observe that its hind-limbs are drawn up under the body; but that it differs from the normal frog in the following respects.

Its head is depressed, instead of being erect.

Its fore-limbs are spread out, or flexed, instead of being held nearly vertical; thus the angle which the body makes with the table is diminished.

There are no respiratory movements, either of the nostrils or of the throat.

2. Gently pull out one of the hind-limbs, until it becomes quite straight, and then let it go. It will be immediately drawn up into its old position under the body. If this experiment be made soon after the operation of removing the brain, or if much blood has been lost, the leg may be drawn up slowly instead of sharply.

3. Gently tickle one flank with a feather or a blunt needle; a contraction of the flank muscles of that side will be observed.

4. Pinch the same spot rather sharply with a pair of forceps; the leg of the same side will be first extended, and then drawn up and swept over the flank, the movement tending to thrust away the points of the forceps.

5. Pinch with the forceps the skin round the anus; both legs will be drawn up and thrust out again; the movement tending as before to sweep away the points of the forceps.

Leave the animal alone for five minutes and watch it carefully: if no disturbing circumstances are brought to bear on it, it will remain perfectly motionless.

6. Place the animal on its back; it will make no effort to regain its normal position, *i.e.* all sense of equilibrium has been lost.

7. Pass a hook through the lower jaw, and fasten it to the cross-bar of a stand so that the body can be raised up and down. The hind-limbs, after a few movements of flexion and extension, will remain pendant and motionless.

8. Gently pinch the tip of one of the toes of either leg; that leg will immediately be drawn up.

9. Fill a beaker with water; and place a little acetic acid .5 to 1 p.c. acid in a watch-glass: let the tip of one of the toes of the frog touch the acid. In a short time the foot will be withdrawn. Dip the foot

into the water, in order to wash away the acid. Measure, with the aid of a rapidly beating metronome, the time between the moment when the toe comes into contact with the acid and the moment when it is withdrawn. Make, at intervals of two minutes, three such observations (being careful that the toe dips in the acid to exactly the same extent) and take the mean of the three.

10. Cut a small piece of blotting-paper one or two mms. square, moisten it with 1 to 5 p.c. acetic acid, and place it on the flank of the animal. The leg of the same side will be speedily drawn up and swept over the flank as if to remove the piece of paper. Wash away the acid.

11. Place similar pieces of acid-paper on different parts of the body; different movements will be witnessed in consequence; all however tending to remove the irritating substance.

12. Wash off all the acid from the frog, and when it has become perfectly quiet, place it in a basin of water; it will sink to the bottom (unless the lungs be accidentally much distended with air), and no movements of any kind will be witnessed.

Observe that all the movements produced in the foregoing observations, although complicated, co-ordinated, and purposeful in character, are partial, and only by accident bring about locomotion. However stimulated, the animal never springs or leaps forward.

In order that the same frog may serve for observations on the lymph-hearts, B. § 1 should be performed here.

13. Make a small cut through the skin of the back, and with a fine glass tube inject one drop of a 1 p.c. solution of sulphate of strychnia. In a few minutes the slightest stimulus applied to any part of the animal will produce violent tetanic spasms of the whole body. A preliminary stage of increased reflex action may also be observed.

14. With a straight seeker or a piece of stout wire destroy the whole of the spinal cord. The spasms immediately cease.

15. Repeat any of the above observations (§ 2—13). No reflex actions will now be produced.

B. AUTOMATIC ACTION.

1. **The Lymph-Hearts.** Placing the animal on its belly watch the movements of the posterior lymph-hearts. They may be seen beating on either side of the extremity of the urostyle, in a depression between that bone and the hip-joint. The contractions are generally visible through the skin, but become more evident if the skin be removed, care being taken not to injure the lymph-hearts themselves.

2. Observe that after destruction of the posterior part of the spinal cord (A. § 14) the lymph-hearts cease to beat.

3. **The Heart.** Expose the heart in a pithed frog, it will be seen in the thin membranous pericardium beating with considerable regularity and force. Pinching up the pericardium with a fine pair of forceps,

cut it away from the surface of the heart, then tilt up the apex of the ventricle; a small thread of connective tissue (the ligament of the ventricle) will be observed passing from the posterior surface of the ventricle to the adjoining wall of the pericardium; cut this through near the pericardium. Lift up the aorta and cut through the aortic branches, the superior venæ cavæ, the inferior vena cava, and the surrounding tissue. Place the heart in a watch-glass, moistening it when necessary with normal saline solution. The beats will either not be interrupted at all or for a very short time.

In cold weather the heart may stop on being removed from the body, but if the heart be warmed by putting the watch-glass containing it in the palm of the hand, the beats will be resumed.

4. Lifting up the apex of the ventricle by means of its ligament, cut through the ventricle with a sharp pair of scissors at its upper third. The lower two-thirds of the ventricle will remain motionless without any spontaneous beat: the auricles and the upper third will continue to beat with regularity.

5. By means of a longitudinal incision divide the auricles with the attached portions of ventricle into two lateral halves. Each half will continue to beat.

6. **Cilia.** Placing the frog on its back, cut through the lower jaw, in the middle line, and carry the incision down the œsophagus as far as the stomach. Pin back the parts divided, and moisten the mucous membrane, if it is at all dry, with normal saline

solution. Place on it in a line as high up as practicable three small thin pieces of cork. The pieces of cork will be seen to be driven by ciliary action towards the stomach; probably the middle piece will travel the fastest.

DEMONSTRATION.

Peristaltic action of the intestine and of the ureters.

LESSON XVIII.

STRUCTURE OF BLOOD VESSELS.

CIRCULATION. INFLAMMATION.

1. **Large Arteries and Veins.** Transverse sections of thoracic aorta, carotid artery, and jugular vein of dog (potassium bichromate 2 p.c.). Stain with hæmatoxylin and eosin (Less. IV. § 6), or with hæmatoxylin and picric acid. Mount together.

a. Note in the aorta the **inner coat**, consisting of an epithelioid lining shown chiefly by the nuclei, and of a thin layer of elastic tissue with a small amount of white fibrous tissue. The elastic tissue shows chiefly as transversely cut fibres.

The thick **middle or muscular coat** consisting of bundles of smooth muscle and elastic fibres circularly arranged in alternating layers.

The **outer coat**, much thinner than the middle, consisting of white fibrous tissue and of elastic tissue; most of the fibres of the elastic tissue are cut transversely, they diminish in number in passing outwards.

b. Note in the carotid artery that the elastic part of the inner coat is chiefly represented by a refractive, and (probably) wavy lamina; and that the elastic tissue is comparatively scanty in the middle coat.

c. Note in the vein that the whole wall is thinner; the inner coat is inconspicuous, except as regards the nuclei of its endothelium, also the middle coat is much thinner than the outer.

2. Cut open longitudinally the jugular, or other large vein of a freshly killed rabbit. Pin it out with hedgehog quills, stream it with .75 p.c. sodium sulphate for a moment, add .2 p.c. nitrate of silver and leave for 10 minutes, wash in distilled water, expose to light for half-an-hour, pass through alcohols, clove oil, and mount in balsam.

Observe the rather jagged dark lines of the cement substance between the cells; the cells form a continuous layer, are more or less elongated in a longitudinal direction, and are flat; indications of their nuclei may be seen, and in some places indications also of the fibres of the muscular coat as transverse or longitudinal markings caused by a deposition of silver in the cement substance between the muscle cells. If the vein has been left too long in the silver nitrate solution, or exposed too long to light, silver will be deposited in the substance of the cells also.

3. Tear off a strip from the inner coat of a medium-sized artery (potassium bichromate .2 p.c. for two to six days; it may be kept in dilute glycerine).

Tease it out in the preserving fluid; it will be found to consist almost entirely of **elastic laminæ**.

Observe the gradations from an almost homogeneous elastic perforated membrane to a meshwork of elastic fibres.

4. Remove a small portion of pia mater from the brain of a recently killed animal, brush it well in normal salt solution, and wash it in more salt solution, make a moist film of it, fix with alcohol, stain with hæmatoxylin (a trace of picric acid after stain is advantageous) and mount in balsam. Note

The smallest arteries; no distinct external coat is seen; the middle coat consists of a single layer of muscle cells wound transversely to the tube, the nuclei are deeply stained, the outlines of the cells will be seen on focussing; the inner coat is represented by the elongated nuclei of the epithelioid lining.

The capillaries showing as thin, nucleated, membranous tubes.

The small veins, of larger calibre than the small arteries, with no muscular coat (this is special to the central nervous system), and in general appearance resembling the capillaries.

5. A pithed frog is given you. Take one or two small pieces of cotton-wool and plug the hole in the vertebral canal. Expose the heart, cutting through the sternum in the middle line, and pinning back the two parts.

Cut away the exposed part of the pericardium, pass a thread under the bulbus aortæ, with fine-pointed scissors make a cut in the bulbus near the ventricle. With a sponge, moistened with normal salt solution, wipe away the blood which comes, stroking the abdomen gently upwards to remove as much blood as

possible. Cut across the inferior vena cava, and sponge up the blood.

Pick up the cut edge of the aortic bulb with fine-pointed forceps, and put into the bulb and up the left aorta a fine nozzled cannula, provided with an inch or so of india-rubber tubing. Tie in the cannula, fill it with .75 p.c. sodium sulphate and pinch the tubing, in order to drive blood out of the nozzle of the cannula, where it rapidly clots.

Fill the cannula again; take a 5 to 10 c.c. injection syringe, the nozzle of which fits the india-rubber tubing, fill it with .75 p.c. sodium sulphate, and push the nozzle into the tubing, pinching the tubing as the nozzle is inserted, so as to avoid air-bubbles.

Inject the sodium sulphate, sopping up with a sponge the fluid which comes from the cut vessels; and now and then stroking the abdomen upwards. When the fluid is nearly colourless (injection of one syringe-full should be sufficient), inject in the same way a syringe-full of 0.2 p.c. nitrate of silver.

Leave for five minutes. Inject a syringe-full of distilled water. Then put the frog in distilled water. Expose the viscera freely, and cut out the following tissues and expose to light in distilled water. The bladder: lift it up by the ends, cut through the membrane attached to it; remove it and cut it open. The intestine and mesentery: lift up the end of the rectum, cut through the mesentery at its dorsal attachment and remove it with the intestine, cut off the mesentery and take the largest pieces; cut open the intestine, scrape off the mucous membrane, and pin out the muscular coat with quills. (The lungs, pinned out, and the kidneys may also be taken; the latter will show the epithelioid cells of the capsules.) When the tissues show signs of reduction, dehydrate pieces—keeping them stretched out—and mount in balsam.

Note the outlines of the flat elongated epithelioid cells of the arteries, of the capillaries and of the veins; the outlines in the capillaries are more irregular than in the arteries or veins; in the veins the cells are rather broader than in the arteries. In the larger vessels the cement substance between the cells of the

muscular coat will also show as black transverse lines, these are more numerous in the arteries than in the veins.

6. Circulation of Blood¹. The frog given you has been deprived of its brain and curarised (cp. Less. XII. § 5). Lay it on its belly on the frog-board, and tie, not too tightly, a piece of soft cotton round the end of the (*e.g.*) 3rd and 4th digits. Stick two pins into the board a little distance from the hole; by twining the cotton round them the web may be stretched out level above the hole (or the toes may simply be pinned out with hedgehog quills, and the quills cut short). Surround the web, and cover the leg and body of the frog with moist blotting-paper. Put a piece of cover-slip over the toes, and with a small brush press the web from below against the slip. (If the web is not in close contact with the glass it may be outside the focal distance of the high objective; in that case, a triangular piece of cover-slip should be placed on the web between the toes, and excess of fluid sopped up, but by this method the front lens of the objective is apt to be smeared by touching the tissue.)

Examine, first with a low and then with a high power. Note .

The course of the blood from the arteries to the veins. A slight pulsation may be observed in the larger arteries and sometimes in the smaller ones.

The greater velocity of the blood in the arteries

¹ In the spring it is best to take a male frog for the circulation in the mesentery (§ 9). The male may be recognised by the wart-like projection on the ball of the thumb.

(owing to their smaller size) than in the veins; probably in neither can the individual corpuscles be made out.

The axial and peripheral zones in the arteries and veins; the peripheral zone is small, and under a low power appears free from corpuscles; under a high power one or two white corpuscles may, if the current is not very fast, be seen in the peripheral zone of the arteries; in that of the veins a few white corpuscles and occasionally a red one will be seen moving along comparatively slowly.

The passage of corpuscles usually in single file through the capillaries;

The elasticity of the red corpuscles; observe the way in which they bend and become deformed, and then regain their normal shape.

7. Inflammation. Remove the cover-slip; compare the circulation in two adjoining webs under a low power. Soak up the fluid on them; on one place a drop of xylol, and examine it, comparing it with the other.

a. The arteries dilate, the veins become larger, and the capillaries much more distinct.

b. At first the circulation is quicker; later it is slower than at first, though the vessels remain dilated.

c. The circulation stops after a time (stagnation), the red vessels being distended with blood (if this is not caused by the xylol, add a drop of turpentine). Where the red corpuscles are pressed together, as in the capillaries, their outline is lost. (For other changes in inflammation cp. § 9.)

d. The effects are local, they are not seen in the other web.

e. If the injury to the vessels has been slight, the circulation may be seen to be re-established in the stagnated spots, the corpuscles gradually recover their outline and are carried off by the current; this is not seen if stasis has set in, *i.e.* if the blood has clotted.

8. Observe now the circulation in the tongue; the frog being on its belly, draw forward the tongue over the hole in the stage and pin out the two cornua; the tongue at first pale soon becomes flushed and its vessels full of blood. With a low power the peripheral zone in the arteries and veins will probably be seen better than in the web.

9. Replace the tongue. On the right side make a cut through the skin and body wall, $1\frac{1}{2}$ to 2 cm. long, midway between the dorsal and ventral surfaces and rather nearer the hind than the fore limbs. Place the frog against the hole in the side of the frog-board, and pull out the coil of the intestine above the rectum, pin it out with hedgehog quills, cut the quills off short, and put small pads of blotting-paper wet with salt solution round the edge of the intestine, cover with a piece of cover-slip, and observe the circulation in the mesentery.

The exposure will probably cause some degree of inflammation, and the stages between the early dilation (§ 7, *a*) and stagnation (§ 7, *c*) may conveniently be followed. Note under a high power

a. The elongated platelets and the spherical white

corpuscles increase in number in the peripheral zone of the arteries and veins, if the current is quick enough to show a peripheral zone; the white corpuscles often roll over along the wall.

b. In the veins, and to a less extent in the capillaries, the platelets and white corpuscles cling to the walls, at first for a time only, later permanently. Here and there a mass of adhering corpuscles forms which stretches across the tube, sometimes this is torn away by the current, sometimes it blocks the vessel and causes stagnation. Note in the stagnated vessels the gradual obliteration of the outlines of the corpuscles.

c. Focus the side of a small vein in which the circulation is slow, and look amongst the white corpuscles adhering to the wall for one which projects a little externally. Draw this at intervals of 15 minutes to note its rate of migration.

10. *Circulation of blood in the newt.* A newt is given you without brain and curarised. *Mesentery.* The hole in the stage should be small, about 1 to 1.5 cm. long, and .5 to .75 cm. broad. The method is the same as in the frog (§ 9).

Pancreas. The skin and abdominal wall are cut through on the left side, the cut beginning a little below the fore leg and extending for 1 to 1½ cm. The pylorus and the part of the intestine below it are pinned out. The circulation in the mesentery is also seen, but less satisfactorily than in the lower loop of the intestine. The same cut allows the lung to be pulled out, and the circulation in it observed.

11. *Preservation of inflamed mesentery.* When the circulation has been observed, cover up with a funnel lined with wet blotting-paper till next day. Remove the lower part of the intestine with its mesentery, and wash with salt solution. Fix the mesentery in alcohol, cut it into two parts, stain one with

Ehrlich-Biondi fluid, and the other with hæmatoxylin and eosin. Mount in balsam. Note the numerous leucocytes with irregularly shaped or fragmented nuclei outside the capillary walls.

DEMONSTRATIONS.

1. Action of valves. A cannula is tied in the peripheral end of a vein (*e.g.* the jugular) of a dead animal. On forcing fluid into the vein it will be seen to swell greatly as far as the proximate valve.

2. Vein cut open and pinned out to show the valves.

3. Longitudinal section of aorta of large animal.

4. Specimen of blood vessels, frog, showing epithelioid cells (*cp.* § 5).

5. Circulation in the lung of the frog or toad (*cp.* p. 130).

LESSON XIX.

STRUCTURE AND ACTION OF THE HEART.

A. HEART OF SHEEP¹.

1. Observe the attachment of the parietal pericardium to the roots of the great vessels.

Remember that the parts of the heart which are right and left in the body are called right and left after removal. The front of the heart may be recognized by a groove filled with fat, the interventricular sulcus, which runs from about the middle of the base of the ventricles to rather below the middle of the right margin of the heart. The front is also more convex than the back. Holding the heart with the front towards you, note that the right ventricle, which will be on your left hand, is much more yielding than the left ventricle, which will be on your right hand. Note also the pulmonary artery arising nearly in the middle line of the heart at the upper part of the ventricles, and immediately behind this the aorta.

¹ The heart should be obtained from the butcher with the pericardium; to secure this it is advisable to purchase the 'bag,' *i.e.* the heart with the lungs still attached to it.

2. Tie a short glass tube into the superior vena cava and connect with it a piece of india-rubber tubing. Ligature the inferior vena cava and the left vena azygos which opens close beside it. Tie a glass tube about two feet in length into the pulmonary artery. Fill the india-rubber tubing with water, and squeezing it press the water onwards. The water will mount in the tube connected with the artery, and will only descend a little way on unclasping the india-rubber tubing. Pour water into the long glass tube by means of a funnel, and observe the column of water which the semilunar valves will sustain. Note the distension of the arterial walls and the bulging at the attachment of the valves. When the pressure of the column of fluid is removed the artery by its elasticity returns to its previous dimensions.

3. Repeat the above observation with the pulmonary veins and aorta.

4. Compare the united sectional areas of the superior and inferior venæ cavæ when distended, with the area of the aorta below the origin of the innominate artery.

5. Having removed the tubes, lay open the superior and inferior venæ cavæ, and bring the incisions to meet in the front of the auricle. Note

The size and form of the auricular cavity. The auricular appendage with its muscular fretwork.

The **septum auricularum**.

The fossa ovalis, or expression of the foetal foramen ovale, which is early closed by the growth of the septum auricularum.

The Eustachian valve, a slightly projecting membranous fold, immediately beneath the entrance of the inferior vena cava, and again beneath this

The opening of the comparatively large left azygos vein.

The auriculo-ventricular orifice.

6. Cut open longitudinally the azygos vein, and observe the **coronary vein**¹ opening into it a very short distance from the heart.

7. Cut away most of the auricle, and holding the ventricle in the left hand, pour water suddenly into the auriculo-ventricular orifice. The **right auriculo-ventricular** or **tricuspid valve** will float up and close the orifice. Note the star-shaped junction of the valve-flaps.

8. Introduce a pair of scissors between two of the valves, and cut through the wall towards the apex. Having arrived at the bottom of the ventricular cavity, turn the scissors sharp round and carry an incision at an acute angle with the previous one, alongside the septum, towards, but not into, the pulmonary artery. Lifting up the flap, note

The thickness of the ventricular wall, the projections of its inner surface or *columnæ carneæ*, the band of muscle (moderator band) running from wall to wall of the ventricle across its cavity. The ventricular cavity does not extend to the apex.

¹ In man, the left azygos vein joins the right, and this runs into the superior vena cava; the coronary vein (coronary sinus) opens direct into the right auricle.

The **tricuspid valve**, its form, and attachment to the auriculo-ventricular ring, the **chordæ tendineæ**, and their attachment to the summits of the papillary muscles.

9. Holding the heart vertically, pour water into the pulmonary artery; observe from below the form of the **semilunar valves**, and their mode of closing.

10. To observe the valves from above, insert into the pulmonary artery a short wide tube, fill it with water, and cover it with a piece of glass, excluding air-bubbles.

11. Prolong the incision of § 8 so as to lay open the pulmonary artery. Note

The form and attachment of the **semilunar valves**.

The small nodule of tissue in the middle of the free edge of each valve, the **corpus Arantii**.

The slight depressions in the arterial walls opposite each valve, the sinuses of Valsalva.

12. Lay open the left auricle in a manner similar to that employed for the right. Note that the left auriculo-ventricular valve, the **bicuspid** or **mitral** has but two flaps. Observe its manner of closing (cp. § 7).

13. Lay open the left ventricle in a manner similar to that employed on the right side, carrying the incision at first along the extreme left of the heart. Note the thick walls, the mitral valve, &c.

14. Lay open the aorta, and examine its semilunar valves, corpora Arantii, and the sinuses of Valsalva,

which are here very distinct. Note that the **coronary arteries** open respectively into two of the sinuses.

B. HEART OF FROG.

1. Expose the heart of a just-pithed frog. With the pericardium intact, observe the pulsations of the heart, noting the alternate beats of the auricles and the ventricle; and the synchronous beats of the two auricles. Lay open the pericardium and observe (best with the aid of a lens)

a. The synchronous contractions of the two auricles, followed almost immediately by

b. The contraction of the ventricle; note that the ventricle during its contraction or systole becomes paler and more conical, and that its apex is thrown forwards and upwards; the obviousness of these changes depends upon the force of the contraction.

c. The slight contraction of the bulbus arteriosus immediately succeeding the ventricular systole.

d. The pause, or diastole, which follows before the auricles again beat; if the heart is beating rapidly this may not be obvious to the eye.

e. The increased redness and distension of the ventricle after the auricular, and immediately preceding its own systole.

2. Divide the ligament of the ventricle (cp. Less. XVII. B, § 3); turn the ventricle forward. Observe

a. The junction of the two superior venæ cavæ with the inferior vena cava to form the sinus venosus.

b. The whitish line, roughly V-shaped, marking the junction of the sinus venosus with the right auricle.

c. The wave of contraction ; it starts in the endings of the great veins, and is most easily seen in the superior venæ cavæ ; then follow in quick succession, contraction of the auricles, the ventricle, and the bulbus arteriosus. Note the distension of the bulbus and the rush of blood through it, as the ventricle contracts.

3. Dissection of the vagus. Make a transverse cut through the skin of the frog just below the lower jaw, and carry the cut as far as the vertebral column. Cut away the skin over the lower jaw. Cut through the superficial muscles connecting the shoulder or sternum with the hyoid bone, the jaw and the skull.

Near the mid line of the lower jaw will be seen two nerves on each side, the hypoglossal and the glossopharyngeal. Near the symphysis of the jaw the hypoglossal—which is the more superficial of the two—lies on the outer side of the glossopharyngeal (cp. Fig. 13). Trace them backwards ; the hypoglossal crosses first the glossopharyngeal and then the branches of the aorta. The glossopharyngeal on coming to the aortic branches runs towards the angle of the jaw, along the upper border of a small band of muscle (part of the petrohyoid). Push a glass tube down the œsophagus into the stomach, and turn the frog on its side. A third nerve, the **vagus**, will be seen on the lower edge of the slip of the petrohyoid muscle mentioned above, and close to the internal jugular vein ; a small branch, the laryngeal nerve, is given off by the vagus, and runs for a time parallel with it. Trace

the vagus to the skull. Pass a needle threaded with a silk thread under the vagus near the skull, tie the thread, cut the nerve close to the skull and isolate it for about a centimetre. Do not isolate near the heart; here it divides into delicate branches for the heart, lungs, and stomach, and these are easily injured.

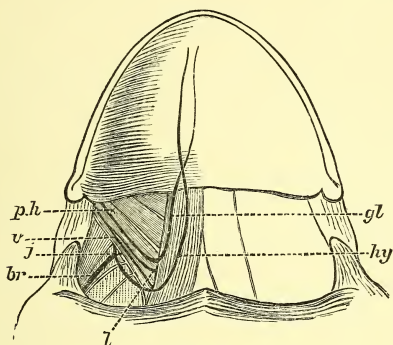


Fig. 13.

p.h., petrohyoid muscle; *gl.*, glossopharyngeal nerve; *hy.*, hypoglossal nerve; *v.*, vagus; *l.*, laryngeal branch; *j.*, internal jugular vein; *br.*, brachial nerve.

Place a piece of thin india-rubber membrane upon the tissue under the vagus and stimulate the nerve with a tetanizing induced current (cp. Lesson XI. A, § 3, *b*) for about 15 seconds. There should be contraction in the larynx, but in no other structure. Watch the heart, and note

a. The **inhibition of the heart-beat**. A second or two after the beginning of the stimulation the heart stops beating, all parts of it being flaccid (diastolic stand-still); and it remains so during the stimulation

and for a short time afterwards. The inhibitory effect varies greatly with the condition of the frog; a slowing and weakening of the heart-beats may follow instead of cessation; and sometimes there is no effect. If no effect is obtained with a weak current, repeat, increasing the strength of the current or dissect out and stimulate the other vagus, the effect of one nerve is often more marked than that of the other.

3. Turn the ventricle forwards and stimulate the line of junction of the sinus and right auricle; the heart will stop beating; there may be a preliminary period of quickened beats caused by the direct stimulation of the cardiac muscle.

4. **Stannius' experiment.** Pass a thread underneath the aortæ, draw it backwards, tilting the ventricle forwards, tie it firmly round the junction of the sinus with the auricle. The auricles and ventricle will cease to beat (there may be one or two beats), the sinus will continue as before. Leave for five minutes, then cut through the junction of the auricles and ventricle; the ventricle will soon or at once begin beating with a slow rhythm, the auricles remaining quiescent.

5. **Graphic record of the cardiac inhibition.** Hold the head of a pithed frog between finger and thumb. Cut away the skin on the back of the head. Pass a scalpel carefully along the posterior edge of the skull, so as to cut through the muscles attached to it. The vagus nerve, the jugular vein, and the petrohyoid muscle will come into view, and on a little further clearing of the muscles, the glossopharyngeal nerve also. Pass a threaded needle under the vagus; put

the frog on its side, tie the nerve and cut it close to the skull. Now expose the heart; cut away the muscles over the vagus, cut off the fore-leg, pin out the frog so that fixed electrodes can be placed under the nerve without touching any other tissue. Obtain a tracing of the movements of the heart in one of three ways.

a. Pass a threaded needle through the extreme tip of the ventricle. Knot the threads about an inch from the heart; hook the thread by a double hook to a lever, 6 to 9 inches long, which has attached to its upper edge a spiral wire spring¹. Arrange so that the writing point of the lever *just* touches the blackened paper of the drum.

b. Tie the ligament of the ventricle, and cut it on the pericardium side of the ligature. Then arrange as in (*a*). By this method the ventricular movements are less fully recorded than in (*a*) but the ventricle is less injured.

c. Leave the pericardium intact. Take a light needle, fixed at the point by wax to a small square of emery paper, and curved at the other. Pass the curved end through a light lever, and let the other end rest on the pericardium over the base of the ventricle. This method causes less injury to the heart than either (*a*) or (*b*) but demands a very light lever.

¹ It is perhaps better to use a lever which projects beyond the fulcrum on the side opposite the writing point, and to attach the thread to the projection; on the latter should be placed a light weight as a counterbalance.

This method is good when the brain only has been destroyed and the frog curarized. If there has been little loss of blood only, the heart fills during inhibition.

Note the form of the ventricular curve with the drum moving fairly rapidly (1 cm. a second). The rise increases at first rapidly, then more slowly to a maximum; the fall is at first slight, then rapid, and finally slow.

Clamp the electrodes in a stand, and place the vagus on the points. The nerve should be touched from time to time with a brush wet with salt solution to prevent it drying.

Stimulate the vagus, and note the tracing obtained.

Stimulate again, keeping the current on; after a variable time the heart will begin to beat notwithstanding the stimulus (escape of the heart).

When the heart begins to beat after vagus stimulation it will for a time probably beat more strongly than before the stimulation. By repeated stimulation the beat will (usually) be considerably improved.

6. *Measurement of latent period.* Arrange a time-marker, marking seconds to write under the other levers; the coils of the time-marker are arranged in circuit with a clock making and breaking contact every second, or with a metronome.

a. Place a time-marker in the primary circuit. Arrange the points of the time-marker and of the heart lever in the same vertical line; with the drum at rest make a vertical stroke with each writing point. Stimulate by closing the key in the primary circuit.

b. Place the time-marker in circuit with a Daniell's cell and a key. Open and close this key simultaneously with the key of the secondary coil and nerve circuit. For this it is best to use Morse keys.

7. Muscarin and atropin. Expose the heart of a pithed frog, cutting through the pericardium. Add one drop of 1 p.c. muscarin, the heart will become slower and stop beating. (If necessary, add a second drop.) Add two drops of 1 p.c. atropin, after a time the heart will recommence beating.

8. *Effect of nicotine and atropin.* Take a tracing of vagus inhibition of a frog's heart by method § 5, c, but opening the pericardium. Let fall on it two drops of 1 p.c. nicotine. In a few minutes stimulate the vagus, no inhibition will be produced, but there may be increase in rate and strength of the heart-beats. Detach the heart from the lever, and stimulate the junction of the sinus and auricle, the heart will stop. Let fall on it two drops of 1 p.c. atropin; and in a few minutes stimulate again, inhibition of the heart will no longer be produced.

9. *Effect of single induction shocks upon the quiescent ventricle.* Stop the beats of the heart by a Stannius' ligature. Connect the ventricle by a thread with a lever writing on a drum. Fix the electrodes so that one is touching one side and the other touching the other side of the ventricle at its middle third. Send a single weak break induced shock into the ventricle, it contracts once. After an interval of a minute stimulate with strong induction shocks, the ventricle contracts once and to the same height as before (*law of maximal response*). Stimulate with a moderate induction shock every five seconds; the successive contractions increase in height for a certain time. (*Stair-case effect.*) Set the drum going at moderate speed, stimulate with a break induction shock, whilst the ventricle is contracting stimulate again, by making the primary circuit, no contraction is produced by the second stimulus (*refractory period*).

10. *Current of action of the heart.* Stop the beats of a frog's heart by a Stannius' ligature. Connect the ventricle by a thread to a lever. Make a pair of non-polarisable electrodes (cp. Less. XIII. § 3) but use straight glass tubes about 5 cm. long, filling half the tube with clay and inserting a worsted thread to

support the projecting clay, which should be moulded on the worsted to a fine point. Put the electrodes in suitable clips and apply one to the base of the ventricle and the other to a point near the apex. Connect the electrodes to a shunt and galvanometer (cp. Less. XIII. § 6). Examine the electrical condition of the quiescent heart by removing the short-circuit plug of the shunt. A deflection will be obtained in the direction which indicates that the apex is negative to the base. The amount of deflection will depend on the extent of injury caused by the thread tied to the apex. If it is small the negativity may be increased by touching the apex with a hot wire. When the spot of light is steady stimulate the ventricle by touching it near the base with a needle. With each beat thus caused a rapid and large movement of the light occurs in the direction indicating that the base becomes negative to the apex. This is the current of action.

DEMONSTRATIONS.

1. Effect of constant current upon the apex of the ventricle.
2. Effect of induction shocks upon the apex of the ventricle (cp. § 9).
3. Rhythmical contraction of ventricle apex under pressure.
4. Blocking of contraction waves in the heart of tortoise.
5. Action of the sympathetic on the heart of frog.
6. The beat of the mammalian heart, and inhibition by the vagus.
7. The stethoscope and the sounds of the heart.

LESSON XX.

BLOOD PRESSURE.

A. MINOR ARTERIAL SCHEME.

This consists of an india-rubber bag, or enema syringe, connected by a tube to a vessel of water and furnished with two valves, one on each side of the bag and opening in the same direction, so that when it is alternately compressed and released by hand water is drawn from the vessel and delivered into tubes beyond the bag. The tubes consist of a piece of glass tubing about 5 or 6 feet long and a piece of rubber tubing of similar length and bore and are connected to the syringe by means of a three-way tube.

There are clamps upon the long india-rubber tube close to its junction with the three-way tube and upon the small piece of india-rubber which connects the three-way tube with the glass tube, so that the flow of water may be through either the glass or the india-rubber tube.

A small piece of india-rubber tubing is also placed on the end of the glass tube, into which a tube finely drawn out can be inserted,

1. Clamp the india-rubber tube at its proximal end close to the pump, and leave the glass tube open so that all the water flows through the latter. Work the pump with a uniform force at about 30 to 40 strokes a minute. To ensure regularity, the strokes had better be timed with a metronome. The water will flow from the open mouth of the glass tube in jerks, corresponding to the strokes of the pump. At each stroke as much will issue from the distal end as enters at the proximal end.

2. Introduce into the open mouth of the glass tube a fine nozzle, so as to offer considerable resistance to the outflow of fluid. Work the pump with the same force and frequency as before. The outflow will still be intermittent, though less fluid will issue from, and consequently less enter into, the tube at each stroke.

3. Clamp the proximal end of the glass tube and unclamp the elastic tube. Let the distal end of the latter be quite open. Work the pump as before. There being little resistance to the outflow, the elasticity of the tube is not called into play, and consequently the flow will be, as in the case of the glass tube, intermittent.

4. Working the pump as before, insert the fine nozzle into the open mouth of the tube. Considerable resistance will now be offered to the outflow of fluid, the elasticity of the walls of the tube will be called into play, and the water will issue from the end of the tube in a continuous instead of an intermittent stream. If the tube be sufficiently long and sufficiently elastic in

proportion to the force and frequency of the strokes, the flow will be uniform as well as continuous.

B. MAJOR ARTERIAL SCHEME.

This consists of a rubber bag or metal syringe with valves, as in the minor scheme, to deliver water into a rubber tube *P* (unshaded in fig. 14) about 30 feet long of about .5 inch diameter. This tube is connected near its distal end by means of **T** pieces and three-way tubes to

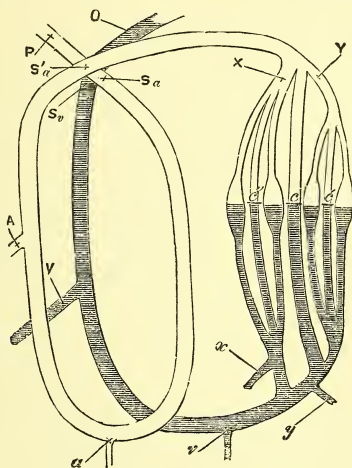


Fig. 14.

two sets of smaller tubes, *x* and *y*, and a tube of equal calibre, *c*. Each set of smaller tubes consists of a straight tube, *c'*, *c''*, and of two dilated tubes, made by introducing in the middle of the rubber tubing a dilated piece of glass tubing. The dilated glass pieces are

stuffed with sponge. The straight tubes can be clamped at c , c' , c'' . All the tubes into which the main tube divides are united again into the single tube O (shaded in fig.) of similar bore to P , and this leads back to the water supply. At A and V side tubes are inserted by which connection can be made to mercury manometers, and at a and v tubes carrying fine nozzles and clamps are introduced.

The pump represents the heart; the small tubes represent the resistance of the small arteries and capillaries. The tubes on the proximal side of this resistance represent the arteries, those on the distal side the veins.

1. The Mercurial Manometer. The manometer A is connected with the arterial, V with the venous tubes.

a. Open the clamps marked c , c' and c'' , so that as little resistance as possible intervenes between the arterial and venous tubes. Bring the manometers to mark on the revolving cylinder, placing V about an inch under A , *in the same vertical line*. Work the pump steadily, regulating the time with the metronome.

In A , the mercury rises at each stroke, and in the interval between each two strokes falls again to its previous level. (The momentum of the mercury frequently carries it below this level, and the descent may be followed by one or more oscillations.)

In V , a similar rise and fall is observed, of nearly if not quite the same extent.

b. Close the clamps c , c' and c'' , so that the capillary resistance becomes very considerable. In A , the mercury

rises rapidly at the first stroke, and at the end of the stroke begins to fall again, but more slowly than was the case in *a*. It has not fallen far before the second stroke raises it to a higher level than before. On falling still again, it is once more raised to a yet higher level, but the increase is not so great as before. Each succeeding stroke has a similar effect. Thus at the end of a few strokes, the **mean arterial pressure** is reached, marked only by comparatively small oscillations corresponding to the strokes of the pump.

On the strokes ceasing, the mercury *gradually* falls until the previous level is reached.

In V the mercury rises to a much less extent than was the case in *a*, a slight mean pressure much less than in A is established, marked either with no oscillations at all or such as are less conspicuous than those of A.

Owing to the presence of the resistance, a mean pressure (arterial blood pressure) is established on the proximal (arterial) side of the resistance. This pressure is marked by oscillations synchronous with the strokes of the pump. On the distal (venous) side the mean pressure is much less and the oscillations are either slight or altogether absent.

2. Flow from Arteries and Veins. Remove the clamps from the fine nozzles *a* and *v*. Let the clamps *c*, *c'* and *c''* remain closed. Set the pump going. The flow from *a* on the proximal (arterial) side is in jets; that from *v* (venous) side is uninterrupted or nearly so.

3. Sphygmograph. Bring the levers S_a (arterial side) and S_v (venous side) to write on the revolving drum, one under the other.

a. Open the clamps c , c' and c'' , and set the pump going. The two levers describe two nearly straight lines, a slight rise only being evident (and that to about the same extent in both) at each stroke.

When there is little or no resistance in the capillaries, comparatively little distension of the arterial walls is produced at each stroke of the pump.

b. Close the clamps c , c' and c'' .

The lever S_a now describes a well-marked curve with each stroke of the pump.

Observe the sudden rise to a maximum, the commencing fall, the break in the fall, followed by a slight rise (dicrotic wave) and the final descent.

The lever S_v describes now a straight line.

The rise in pressure at each stroke indicated by the mercurial manometer is accompanied by a distension of the proximal (arterial) part of the tubing, indicated by the rise of the lever. This is the pulse.

On the distal (venous) side of the resistance no pulse is visible.

4. Progression of the Pulse-wave. Place two levers, one S_a , as near as possible to the pump, the other S'_a , as near as possible to the resistance. Bring the two levers to mark on the cylinder *the one exactly beneath the other.*

(The pressure exerted by the two levers must be as nearly equal as possible.)

Observe that each rise of S_a begins a little before, and is over a little before that of S'_a . In other words, the pulse of S'_a is a little later than that of S_a .

Measure by means of a tuning-fork and time-marker

(cp. Lesson XI. C. § 3) this interval of time, and the length of tubing between the two levers being known, calculate the rate of progression of the pulse-wave.

5. While the pump is working, the clamps being closed and the manometers A and V tracing their curves, *gradually* diminish the resistance by opening slowly first c' and then c'' .

The arterial pressure curve will gradually fall, still marked by the pulse oscillations; the venous curve will gradually rise.

Diminution of capillary resistance lowers arterial, but increases venous pressure.

6. Close the clamps c' and c'' , and take tracings with the manometers, then gradually reduce the strength of the strokes of the pump.

Both arterial and venous pressure will diminish.

7. The clamp c being closed, the main arterial trunk of the scheme divides into two chief branches, X and Y, each with its own resistance and venous tube.

Leave the clamps c' , c'' closed, and put clamps on the tubing immediately beyond x and y .

a. Work the pump with great regularity, and measure the quantity of fluid which escapes during a given time (say ten seconds) from the venous tube of X, and from that of Y, by the side tubes x and y .

b. The clamp c'' of X remaining closed, open c' of Y, and the pump working exactly as before, measure again the outflow during ten seconds.

The outflow of Y will be increased. That of X on the other hand will be diminished, though the resistance in X is the same as before.

The flow of blood through an artery is dependent not only on the resistance offered by its own small arteries and capillaries but also on that of other arteries.

DEMONSTRATIONS.

1. The effects in the rabbit on the temperature of the ear, and on the calibre of its blood vessels, following

a. Stimulation of the central end of the great auricular nerve.

b. Section of the sympathetic nerve in the neck.

c. Stimulation of the peripheral end of the sympathetic.

2. Normal kymographic tracings of the blood pressure of a mammal obtained by the use of a mercurial manometer.

3. The effects on the arterial blood pressure, as indicated by the tracing, produced by

a. Inhibition of the heart through stimulation of the peripheral end of the vagus.

b. Dilatation of the small blood vessels through stimulation of the central end of the depressor nerve.

4. Methods of measuring the velocity of the blood current in large vessels.

5. Comparison of venous and arterial pressure.

6. Method of using the sphygmograph.

7. Method of using the cardiograph.

LESSON XXI.

SALIVARY GLANDS. CERTAIN CARBOHYDRATES. SALIVA.

A. SALIVARY GLANDS.

1. **Albuminous glands.** Tease out a *small* fragment of the parotid gland of a recently killed mammal, *e.g.* of a rat, in a small drop of normal salt solution. Cover, press lightly, and examine under a high power. The outlines of the alveoli and cells are faintly seen; the nuclei are not seen, except perhaps in some of the peripheral alveoli, altered by the salt solution; note the numerous **granules**. Fix the cover-slip (first sopping up excess of salt solution, if the cover-slip moves easily when touched), and observe the crowded granules in the fluid.

2. Section of albuminous gland. (Parotid, or sub-maxillary gland of rabbit. Chromic acid and alcohol mixture; picro-carmin.) Observe

a. Under a low power,

The **alveoli**, appearing as small roundish, or short tubular bodies, closely aggregated together to form the lobules; each will be seen to consist of a group of cells

surrounded by a small amount of connective tissue. The small **ducts** chiefly in the centre of the lobules, cut obliquely and transversely.

b. Under a high power,

The alveoli vary in size; externally the basement membrane shows as a rather sharp outline; the lumina are small and may not be visible, unless kept open by secretion. In the round alveoli—tubes cut transversely—it will be seen that there is one layer of cells only between basement membrane and lumen.

The cells are more or less polyhedral; they have an irregularly granular appearance, but the cell-granules, seen in the fresh gland, have disappeared. The nuclei are spherical (unless shrunk by the treatment), the nucleus of each cell is placed a little nearer the outer than the inner side of the cell.

The **ducts** consist of a single row of slender columnar cells, the inner borders of which form a distinct ring bounding the obvious but small lumen; the outer boundary is not marked by a sharp line. The outer part of the cells is striated (it stains readily with picric acid). The nucleus is ovoid and situated at about the inner third of the cell.

3. *Section of lachrymal gland of a rabbit.* (Mercuric chloride; hæmatoxylin.) Note that the terminal secreting tubes are obviously tubular, they will probably show distinction between an outer, fairly homogeneous, stained zone, and an inner, irregular granular, unstained zone. (The zones can be readily seen in the fresh gland.)

4. **Mucous glands.** Tease out in 2 to 5 p.c. NaCl a piece of the orbital or sub-maxillary gland of a

recently killed dog¹. Observe the distinct (mucous) granules at the edges of the specimen. Irrigate with HCl 1 p.c.; the granules swell up and disappear; here and there a pear-shaped mucous cell will be seen with a nucleus and small amount of protoplasmic cell substance at the narrow end, and a network of cell substance stretching through the swollen, mucous portion.

5. Section of dog's resting sub-maxillary gland (alcohol; picrocarmine, or better, Ehrlich-Biondi fluid). Note under the low power that the general features are the same as those of albuminous glands § 2 (a).

Note under a high power that two kinds of cells are present in the alveoli, the mucous cells and the demilune cells (the latter are not present in all mucous glands).

The **mucous cells** are comparatively large, and have rounded outlines; most have a disc-shaped nucleus close to the basement membrane; in a few the nucleus is spherical and farther from the basement membrane. Much the greater part of the cell is clear and homogeneous, consisting of mucin (or mucigen) resulting from the running together of the mucous granules (cp. § 4). The mucin is unstained in the carmine specimens². The protoplasmic cell substance is arranged much as in the cell swollen by acid (cp. § 4).

¹ It is best to take the orbital gland from the body a day after death; isolated mucous cells more or less columnar and full of granules may then be obtained, and the nuclei and nucleoli may also be visible.

² The mucous part of the mucous cells does not stain with hæmatoxylin, unless hardened in acid reagents; it stains deeply with thionin.

The **demilune cells** occur usually in half-moon shaped groups, at the ends of the alveoli; they are more or less overlapped by the swollen mucous cells. The outlines of the several cells are not very distinct. The nuclei are spherical, the cell substance, which stains equally throughout, resembles that of the cells of the albuminous glands.

The ducts are in general features like those of the albuminous glands (cp. § 2).

6. **Active mucous gland.** Section of dog's sub-maxillary gland after prolonged secretion¹. The section should be stained in the same way as that of § 5. Observe, comparing it with the resting gland of § 5 (it is best to mount the two sections under the same cover-slip), the mucous cells are smaller, owing to a diminution in the mucous part of the cell and have less rounded outlines; the mucin which remains still borders the lumen. The protoplasmic cell substance is more abundant. The nuclei are spherical, have conspicuous nucleoli, and are farther from the basement membrane.

The alveoli are not all changed to the same extent; in some the mucin has almost entirely disappeared, and the demilune cells are more polyhedral, so that the two kinds of cells are not very easy to distinguish; in others the only changes observable are that the nuclei of the mucous cells are spherical and the demilunes more conspicuous.

7. Tease in 5 p.c. neutral ammonium chromate a piece of a dog's sub-maxillary gland which has been kept for 3 to 6 days in

¹ In a dog under morphia and chloroform, the chorda tympani (or this with the sympathetic) is stimulated at short intervals for three to six hours.

the fluid (or for 3 days in 2 p.c. chloral hydrate). Observe the isolated mucous and demilune cells. The general appearance of a mucous cell is like that produced by dilute HCl in § 4, but the basal end of the cell is seen to be prolonged into a process.

8. Sections of dog's orbital gland hardened in osmic acid vapour, given out in paraffin.

(a) Stain some sections on a cover-slip with alcoholic methylene blue. Observe the deeply stained mucous granules stretching throughout the cells. The protoplasmic cell substance is stained greenish.

(b) Place a section in xylol to dissolve the paraffin, transfer to absolute alcohol, mount in 95 p.c. alcohol. Observe the brownish mucin granules. Irrigate with 50 p.c. alcohol, watching the cells carefully.

The granules will be seen to swell up till their outlines are lost, the cells swell, their outlines become rounded; an intracellular network becomes visible; thus the general appearance of the hardened gland of § 5 is obtained. Transfer to absolute alcohol and the original appearance will return. (If the tissue has been too short a time in osmic acid vapour the granules as they swell may stick together, in this case absolute alcohol brings back the original appearance imperfectly or not at all.)

B. REACTIONS OF CERTAIN CARBOHYDRATES.

1. To a few c.c. of **starch** mucilage¹ 1 p.c. add a drop or two of a moderately strong solution of iodine; an indigo-blue colour will be produced; if the colour is very dark fill up the test-tube with water.

¹ To prepare the starch mucilage take 1 gram of starch and rub it into a thin paste with cold water. Pour it into a beaker containing one hundred c.c. of boiling water, boil for a few minutes and place it aside to cool. It should have no lumps in it and should be thin enough to be measured out readily with a pipette.

2. To a 2 p.c. aqueous solution of **dextrin**¹ add a strong solution of iodine, drop by drop. A deep brown-red colour will be produced. Warm; the brown-red colour will rapidly disappear, a light brownish-yellow tint due to the iodine remaining; on cooling, the dextrin colour returns. Now add water; as the dextrin solution becomes more dilute, the red tint becomes less obvious, the fluid appears yellow-brown. That this colour is due to the dextrin can be seen by warming the fluid, and noting its change of tint.

3. To 5 c.c. of a (.1 p.c. solution) of **dextrose** (grape-sugar) add an excess of a solution of sodium hydrate (5 p.c.) and a couple of drops of a 1 p.c. solution of cupric sulphate; the precipitate of hydrated cupric oxide at first formed will dissolve, giving a blue solution. Boil; the cupric oxide will be reduced and a yellow or red precipitate of cuprous oxide will be produced (**Trommer's test**). When a very small quantity of sugar is present no distinct precipitate is obtained, but the fluid is decolorized or turns faintly yellow. Repeat this, adding half-a-dozen drops of a strong solution of cupric sulphate; the reaction will be much less obvious, partly owing to the blue colour of the dissolved hydrated cupric oxide and partly to the brown-black precipitate of anhydrous cupric oxide.

4. Add to a solution of dextrose some strong NaHO and boil; the solution turns yellow, yellowish-brown, or nearly black according to the amount of sugar present (Moore's test).

¹ This may be bought or it may be prepared by boiling a little starch with sulphuric acid about 3 p.c., until a drop of the fluid gives a red-brown colour with a drop of iodine.

5. Add to a solution of dextrose some sulphindigotate of soda solution and some Na_2CO_3 and boil; the blue colour turns green, reddish purple, red and yellow. When shaken with air the blue reappears (Mulder's test).

6. Add to a solution of dextrose an equal vol. of saturated aqueous solution of picric acid, and some caustic soda; boil. The solution becomes very dark red.

7. Add to a solution of dextrose half its vol. of acetic acid and 2 or 3 drops of phenylhydrazin; place the test-tube in a beaker of water and boil for $\frac{1}{2}$ -hour. On cooling, if not before, yellow crystals of phenyl glucosozone separate out. Examine the crystals microscopically. They are acicular and occur singly, in bundles and in star-like clusters.

8. **Cane sugar.** To a 1 p.c. sol. of cane sugar apply Trommer's test; no reduction occurs. Add strong HCl to the cane sugar solution, 1 c.c. for each 10 c.c. of sol. taken, and boil gently for 10 min. The cane sugar is converted into lævulose and dextrose. Cool the solution, neutralize it, and apply Trommer's test.

9. **Quantitative estimation of dextrose.** *Preliminary estimation.* Put the solution of dextrose in a burette, let a few drops escape, so that the nozzle is filled and read the level of the solution. Into a small porcelain dish measure accurately 10 c.c. of Fehling's fluid (see App. p. 374), add about 10 c.c. of sodium hydrate and about 30 c.c. of water; boil; as soon as it boils run in the sugar solution from the burette in such amount that the fluid does not cease to boil, and stir continuously. When the fluid undergoes a distinct change of colour add the solution more slowly, and as soon as the fluid turns bright yellow or brick red remove the flame for a few moments and allow the

precipitate partially to settle. Gently tilt the dish and observe if the fluid is still distinctly blue. If it is add a few more drops of the sugar solution and boil again. Proceed in this way until the fluid is no longer distinctly blue. Note the amount of sugar solution run in. Since 10 c.c. of Fehling is reduced by $\cdot 05$ gram of sugar, the percentage of sugar will be $\frac{\cdot 05 \times 100}{b}$, where b is the number of c.c. run in.

There are certain sources of error in the method given above. (1) The cuprous oxide takes up oxygen from the air and a cupric salt is formed, giving a blue tinge to the solution. The re-oxidation of the precipitated oxide may be lessened by boiling in a small glass flask instead of in an open porcelain dish, but since the former is more easily cracked during boiling, and in it the colour of the fluid is less readily seen, it is not recommended for the beginner. In any case the estimation should be made as quickly as possible. (2) When the solution contains more than 1 p.c. or less than $\cdot 5$ p.c. of sugar, the method is inaccurate; since 5 c.c. of a 1 p.c. solution of sugar, or 10 c.c. of a $\cdot 5$ p.c. solution contain $\cdot 05$ gram of sugar, *i.e.* will reduce 10 c.c. of Fehling's fluid, the solution, for an accurate estimation, must be diluted to such an extent that not more than 10 and not less than 5 c.c. of the diluted solution are required to reduce 10 c.c. of Fehling's fluid. The Fehling's fluid is diluted 5 times because with either a greater or a less dilution the results are inaccurate.

Second estimation. If the solution is found to contain more than 1 p.c. of sugar, dilute it accurately with a certain number of volumes of water, to bring its percentage amount within the limits given above, and calculate how much of the diluted solution will probably be required to reduce 10 c.c. of Fehling. Even if no dilution is required make a second estimation, working quickly. Boil 10 c.c. of Fehling diluted as before and

run in all at once about 1 c.c. less than the amount of sugar solution which will be required. Boil, then remove the burner and observe the colour of the fluid, and finish the estimation by adding the solution a few drops at a time.

C. SALIVA.

1. Look at a little fresh saliva under the microscope (h. p.). A few swollen spherical leucocytes, their granules showing Brownian movement, and some flat epithelium cells from the mucous membrane of the mouth will probably be seen.

2. Test with neutral litmus paper the reaction of a drop of saliva, it will be found to be alkaline.

3. Induce the secretion of saliva by chewing a small piece of india-rubber tubing, or by rubbing the tongue with a crystal of tartaric acid.

If time allows let the saliva stand until the turbidity has settled down into a sediment. To a few c.c. of the fluid add strong acetic acid; **mucin** will separate out as a stringy mass, which does not dissolve in excess of acid (cp. Less. x. § 6, *a*). Shake gently, or stir it with a glass rod, the mucin will form a clump; remove it, and if the fluid is cloudy, filter.

4. To the clear fluid add a drop or two of a strong solution of potassium ferrocyanide. The slight precipitate which results indicates the total quantity of **proteids** present (cp. Lesson III. *e*). If the reaction is not obvious, test another small portion with Millon's reagent.

5. In this and the following experiments the saliva¹ used should be diluted 5 to 10 times.

Mix equal quantities (say 5 c.c.) of starch and saliva in a test-tube and place in a water bath at about 37° C. At short intervals (1 to 3 minutes) take a drop of the mixture and add it to a drop of iodine on a porcelain plate. The blue colour produced at first will later become a blue-violet, a red-violet, a red-brown, and a light-brown yellow, according to the relative amounts of starch and dextrin present, finally there will be no coloration, no more starch or dextrin (erythrodextrin) being left. Then divide the fluid into two parts.

a. Add iodine; no coloration is produced (there may be a little tint from dextrin, since in mixing the drops a faint colour may escape notice which in a larger quantity of fluid is obvious).

b. Add an excess of sodium hydrate and a drop of 1 p.c. cupric sulphate and boil; the fluid turns yellow and a yellow or red precipitate will be formed, showing the presence of sugar.

6. Boil a little saliva, add it to starch in a test-tube and warm. In half-an-hour divide into two parts and test as in § 5, *a*, *b*. The blue colour from starch will be as distinct as at first, no trace of sugar will be found;

¹ An aqueous extract of a ptyalin-containing gland may be used instead of saliva. To prepare the extract take (*e.g.*) the parotid glands of a rabbit and having removed the connective tissue around them chop them up well and place the pieces in about 200 c.c. of water; leave in the warm for an hour or two and filter. The aqueous extract thus prepared contains much proteid material, and this obscures the reducing action of sugar on cupric hydrate in Trommer's test when a small quantity only of sugar is present.

hence boiling destroys the ferment (ptyalin) which converts starch into sugar.

7. If the saliva used in § 5 converts starch into sugar very rapidly, dilute it still further for the following experiment. Into each of three test-tubes pour equal quantities of saliva and starch. Place *A* in a water bath at about $37^{\circ}\text{C}.$ ¹, leave *B* at the temperature of the room (noting it), and place *C* in a vessel with ice (it is best to cool the starch and saliva before mixing them). At short intervals take with a glass rod drops from each and add them to drops of iodine on a porcelain plate and so compare the rate of disappearance of starch (cp. § 5) in the three mixtures. It will disappear much more quickly in *A* than in *B*; in *C* there will be very little change.

When no starch is left in *A*, remove *C* from the ice and place it in the warm chamber and test at intervals as before, the starch soon disappears. *Hence a temperature of $0^{\circ}\text{C}.$ arrests the action of saliva but does not destroy it.*

8. Neutralize a small quantity of saliva; to 5 c.c. of this add 5 c.c. of HCl $\cdot 2^2$ p.c., the mixture thus contains $\cdot 1$ p.c. HCl . Place at $37^{\circ}\text{C}.$ for ten minutes, add 3.5 c.c. Na_2CO_3 $\cdot 4$ p.c. and complete the neutralization with a more dilute solution. Add a few c.c. of starch and place at $37^{\circ}\text{C}.$ In half-an-hour test for starch and sugar; starch will be found but no sugar, hence the acid has destroyed the ptyalin.

¹ In these and in similar experiments $37^{\circ}\text{C}.$ is taken, since that is very nearly the normal body temperature of man, but a rather higher or a rather lower temperature will serve equally well.

² Pure strong commercial hydrochloric acid contains about 33 p.c. HCl .

9. Place in one dialyser¹ (*A*) 15 c.c. of starch and in another (*B*) 10 c.c. of starch with a little saliva.

Test from time to time the external water in each. That from (*A*) will give no trace of starch or sugar. That from (*B*) will contain sugar but no starch. Sugar dialyses, but starch does not.

10. Add a little raw starch (or arrow-root) to saliva and place in the warm chamber, shaking frequently. The raw starch is converted into sugar very slowly, it may be an hour or more before any sugar can be detected.

11. Apply a drop of saliva to filter-paper which has been dipped into a weak solution of ferric chloride acidified with hydrochloric acid and allowed to dry. A brownish-red stain on the paper will probably be produced—with human saliva—indicating the presence of a sulphocyanate.

DEMONSTRATIONS.

1. Transverse section of the hilus of the submaxillary gland of a cat or dog. Note; the branching duct with large lumen and one or two layers of cubical or flattened epithelium cells; close to the duct groups of nerve-cells forming part of the submaxillary ganglion; the gland artery, and probably a vein.

2. Section of dog's orbital gland to show the mucous granules (cp. § 8, *a*).

3. Section of dog's active submaxillary gland hardened in osmic acid vapour to show the inner granular and outer protoplasmic zones.

¹ A very convenient dialyser may be made from a short length of parchment paper tubing (Papier-Därme) sold by Carl Brandegger, Ellwangen, Württemberg.

LESSON XXII.

STOMACH. GASTRIC JUICE. NUCLEO- PROTEIDS. MILK.

A. ŒSOPHAGUS AND STOMACH.

1. Transverse vertical section of the **cardiac end** of the **stomach**¹ (alcohol or chromic acid ·2 p.c.). Stain with hæmatoxylin and eosin.

a. Observe under a low power,

Externally, the thin connective-tissue layer of the peritoneum.

The **muscular coat**, consisting of an outer longitudinal and an inner circular coat of unstriped muscle, the former will appear as a cross section of a number of bundles with connective tissue running in between them from the peritoneum; the latter as a continuous layer. On the inner side of the circular coat some small oblique muscular bundles may perhaps be present.

The **submucous coat** of connective tissue. If the mucous membrane is in folds the submucous but not the muscular coat will be seen to run up in the folds.

¹ Stomach of rabbit, cat, or dog. The muscular layers are thinner in the rabbit than in the cat or dog.

The **muscularis mucosæ**, or thin stratum of unstriated muscle fibres a little external to the glands, this is divided more or less distinctly into an outer longitudinal and an inner circular layer.

The **mucous coat**. Note in this the **oxyntic gastric glands** with their openings and the ridges between the openings. The bifurcation of some of the glands will probably be made out.

b. Observe under a high power,

The columnar **mucous cells**, lining the mouths of the glands and covering the free surface of the mucous membrane between them; they are long, slender cells, becoming shorter in passing down the mouths of the glands; the upper third of the cell (containing mucin) is much more transparent than the remaining portion; the nucleus lies at about the lower third. (The stomach must be hardened very soon after death, or these cells will be detached.) An oblique section of the mouths of the glands will cut through two or more of the columnar mucous cells on each side of the lumen, thus a number of small polygonal areas may be seen in the gland mouth, sometimes apparently blocking it up.

The large deeply stained **ovoid** or **oxyntic cells** with ovoid nuclei, and the short columnar or polyhedral **central** or **chief cells** with spherical nuclei. At the base of the glands the central cells are usually most numerous, the ovoid cells being placed between them and the basement membrane; towards the neck of the glands the ovoid cells increase in number; in the neck, the majority of the cells are ovoid, and abut on the lumen. The ovoid cells usually cause a bulging out-

wards of the basement membrane, this is especially the case if the animal has been killed soon after it has fed.

The connective tissue immediately internal to the muscularis mucosæ; it surrounds the bases of the glands, and sends up processes between them. It runs between the glands,—generally with a few muscle-cells from the muscularis mucosæ;—and is seen as thin bands between the bodies of the glands; the bands spread out near the surface. Leucocytes are present, but not in great numbers.

2. Section of the fundus region of the gastric mucous membrane, through the bodies of the glands parallel to the surface. Observe

The central cells forming a tube with very small lumen.

The comparatively rare ovoid cells on the outer side of the central cells.

3. Cut with scissors a thin strip of the fundus mucous membrane of a recently killed guinea-pig or rabbit. Mount it without fluid and gently press the cover-slip. Note in the deeper parts of the glands the central mass of granules (granules of the central cells), and the projecting ovoid cells without distinct granules. Irrigate with salt solution, the granules of the central cells in time disappear, and the ovoid cells become irregularly granular. (The central cell granules are not preserved by most hardening agents, cp. § 1.)

4. Vertical section of the **pyloric end** of the stomach. (Alcohol; hæmatoxylin.) Compare these with the sections made of the cardiac end. Note

The greater thickness of the muscular layers.

The wider and longer mouths to the **pyloric gastric glands**, their more frequent branching, the absence of ovoid cells (if the section passes through the upper part of the pyloric region a few ovoid cells may be seen), the short columnar cells of the bases of the glands and the usually distinct lumina.

5. Examine fresh pyloric glands (cp. § 3). The cells are devoid of the distinct granules seen in the central cells of the fundus.

6. Section of cardiac region of stomach of frog (osmic acid). Observe that the surface cells are in general features like those of the mammalian stomach; the necks of the glands have swollen mucous cells; the bodies of the glands have one kind of cell only, in shape somewhat resembling the ovoid cells, but containing distinct granules. (In lower vertebrates the granules of the fresh gland are preserved by osmic acid.)

7. Transverse vertical section of the lower third of a rabbit's **œsophagus** (potassium bichromate 1 p.c.). Stain with hæmatoxylin (or Ehrlich-Biondi fluid) and compare it with the corresponding sections of the stomach. Note the following points of contrast:

The muscular coat contains striped as well as unstriped muscular fibres; sections from the upper part of the œsophagus show no unstriped fibres.

The submucous tissue contains small albuminous and mucous glands (cp. Lesson XXI.). Each of these consists of a duct, dividing and ending in dilatations, the alveoli.

Traces of the muscularis mucosæ internal to the alveoli of the glands.

The papillæ of the mucous membrane.

The epithelium forming a layer several cells deep, the deeper being columnar or spheroidal, the superficial cells flattened (cp. Epidermis, Lesson XXXII).

B. GASTRIC JUICE.

1. *Artificial Gastric Juice.* *a.* Tear off the mucous membrane from the stomach of a mammal, cutting away the pyloric region (the stomach of a pig obtained from the butcher's will serve). Mince it finely. Put it in a flask with two hundred times its bulk of hydrochloric acid $\cdot 2$ p.c., and place the flask in a water bath at about 40° C. After some hours a considerable part will be dissolved. Decant, and filter the decanted fluid. A solution of pepsin in hydrochloric acid will be obtained; it will, however, contain a considerable quantity of peptone.

b. Mince another gastric mucous membrane; remove with blotting-paper the excess of fluid, add five times its bulk of glycerine and place aside, stirring occasionally. It is best to leave the mixture for some days before use, it may be kept almost indefinitely. When required for use filter through muslin, add to the fluid ten to twenty times its volume of HCl $\cdot 2$ p.c. and filter.

2. **Action of Gastric Juice.** Use a peptic extract prepared as above or take 5 grams of commercial pepsin to 1000 c.c. of HCl $\cdot 2$ p.c. and dissolve by rubbing up the pepsin with a few drops of the acid in a mortar, then add the rest of the acid and if necessary filter.

a. Take four test-tubes. In *A* place 5 c.c. of hydrochloric acid $\cdot 2$ p.c. In *B* 5 c.c. of the peptic extract. In *C* 5 c.c. of the same fluid, carefully neutralized with dilute Na_2CO_3 . In *D* 5 c.c. of the same fluid thoroughly boiled. Add the same quantity of

fibrin¹ to each, and place in a water bath at about 37°C. Examine from time to time:

A, the fibrin will swell up and become transparent, but will not be dissolved; on neutralization it will appear unaltered.

B, the fibrin will be digested.

C, the fibrin will be unaltered.

D, the fibrin will be like that in *A*.

These experiments show that acid alone (*A*) and pepsin alone (*C*) will not digest fibrin, and that pepsin loses its power on being heated to boiling point (*D*). Now add acid again to *C*, and place it in the warm chamber. Digestion will take place. The neutralization has only suspended, not destroyed, the action of the pepsin.

b. Take two test-tubes, with 5 c.c. of peptic extract and a morsel of fibrin in each.

Place *A* in the warm. Surround *B* with ice, or put it in a cold spot.

The fibrin in *A* will be digested rapidly; that in *B* very little or not at all.

3. Take 5 c.c. of peptic extract which has been found to digest fibrin rapidly, neutralize it, filter and add an equal bulk of Na_2CO_3 2 p.c., thus obtaining pepsin in the presence of a small quantity of an alkaline

¹ Raw fibrin digests more easily than that which has been boiled or kept in alcohol, it often however contains traces of pepsin so that a slow digestion may take place when acid only is added to it. When it is required to measure accurately the amount of fibrin added, raw fibrin finely chopped up should be placed in dilute HCl until it is well swollen, the excess of acid poured off and the fibrin measured in small tubes containing (*e.g.*) 2 c.c.

salt. Place at about 40°C . for half-an-hour to an hour. Then add HCl until the mixture is distinctly acid (or neutralize and add an equal volume of HCl 4 p.c.). Add a flock or two of fibrin and warm. Little or no digestion will take place. The pepsin has been destroyed by the alkaline salt.

4. **Preparation of peptone.** Place 50 c.c. of peptic extract together with some fibrin or other proteid in a beaker and leave in the warm until a small part only of the proteid remains undissolved. Filter and neutralize carefully, a precipitate of acid albumin (para-peptone) will be obtained (cp. p. 81). Filter off the acid albumin, the filtrate contains peptones.

Since all samples of commercial pepsin contain peptone, the detection of peptone after a digestion experiment does not indicate that peptone has been formed.

5. Determine the following **characters of peptones** with the solution obtained in § 4; or with a 1 p.c. sol. of commercial peptone¹.

a. Apply the tests for proteids (cp. p. 20), Millon's and the xanthoproteic reaction are obtained, but no precipitate is produced with acetic acid and potassium ferrocyanide.

b. Add excess of sodium hydrate and a drop of *dilute* cupric sulphate, a rose colour is produced (biuret reaction); a slight excess of cupric sulphate obscures

¹ Commercial "peptone" consists largely of albumoses.

the rose colour, turning the tint to violet or mauve. (Cp. Lesson III. § 4, c.)

c. Boil; it does not coagulate.

d. Pour into one dialyser (*A*) a solution of peptone and into another (*B*) diluted serum or white of egg, having only a small quantity of water outside the dialyser. Leave for an hour or longer, then apply the xanthoproteic test to the fluid outside the dialyser, a reaction will be obtained from (*A*) only, *i.e.* the peptone has dialysed, the albumin has not.

6. *Preparation of albumose (hemialbumose).* Take about ten grams of fibrin, chop it finely, warm it in a beaker with three or four volumes of HCl .2 p.c. till it is swollen up. Pour off excess of fluid. Add to it about $\frac{1}{10}$ of its volume of a strong peptic extract and place in the warm. In about 30 minutes the fibrin should be almost completely dissolved. Then add dilute sodium carbonate until the reaction is neutral or faintly alkaline. A bulky precipitate, chiefly of acid albumin, is formed. Filter. The filtrate contains hemialbumose and but little peptone. Apply to it the following tests¹.

a. Cool the test-tube containing it, then add a few drops of nitric acid, a precipitate is formed which disappears on warming and reappears on cooling.

b. Add a few drops of acetic acid and a drop of potassium ferrocyanide, a precipitate is formed, which disappears on warming and reappears on cooling.

c. Acidulate with acetic acid, and add NaCl to saturation, hemialbumose is precipitated.

d. Saturate with ammonium sulphate, the albumose is precipitated.

¹ The hemialbumose reactions may be obtained with a solution of Witte's peptone.

e. Filter off the precipitate from *c* or *d*, dissolve it in a little water. Apply the biuret test, a rose colour is obtained as with peptone § 5, *b*.

f. Take the filtrate from *d* and apply to it the biuret test, adding excess of NaHO, *i.e.* twice its volume of 40 p.c. NaHO, a rose colour indicates peptone.

C. NUCLEO-PROTEIDS.

1. *Preparation.* (*a*) Take lymphatic glands of the ox, free them as much as possible from fat, mince, grind up with sand and extract for 1 to 2 hrs., with 10—20 times the volume of water, shaking frequently, strain and centrifugalise. To the fluid add acetic acid drop by drop until distinctly acid, a bulky precipitate of nucleo-proteid is formed. Let it stand for a few minutes, best in the warm, then collect the precipitate by centrifugalisation, dissolve it in 1 p.c. Na_2CO_3 . Purify by reprecipitating it with acetic acid.

(*b*) Grind up minced lymphatic glands with an equal volume of solid NaCl. Throw the material into a large volume of water, nucleo-proteid rises to the surface in stringy masses, collect it.

2. **Properties of nucleo-proteid of lymphatic glands.** *a.* It dissolves in dilute alkalis and alkaline salts, forming a slightly opalescent solution. Make a solution in 1 p.c. Na_2CO_3 .

b. Boil the alkaline solution; no coagulation occurs. Boil nucleo-proteid in suspension in water or in slightly acid fluid, it coagulates and is then comparatively insoluble in dilute alkali.

c. Apply to a solution of nucleo-proteid the general proteid reaction; all reactions are obtained.

d. Add magnesium sulphate or ammonium sulphate to saturation; the nucleo-proteid is precipitated.

e. Add acetic acid drop by drop, nucleo-proteid is precipitated when distinctly acid and is not readily soluble in excess of the acid.

f. Add precipitated nucleo-proteid to a pepsin solution in .2 HCl, and place in the warm for 3—4 hrs. An insoluble residue of nuclein remains. Filter or centrifugalise off the residue. Boil it in strong nitric acid and add a few drops of ammonium molybdate, a canary-yellow colour, indicating phosphorus, is obtained.

D. MILK.

1. Examine a drop of fresh cow's milk under the microscope with a high power. It consists of a clear fluid containing a large number of highly refractive **fat globules** of varying size.

2. Test the reaction of fresh cow's milk with litmus paper. It will be found to be **alkaline**: occasionally it is acid, owing to the presence of free lactic acid.

3. Dilute a little milk five to ten times with water; neutralise it cautiously with strong acetic acid, no precipitate will fall. Continue to add the acetic acid drop by drop, a copious precipitate of **caseinogen** will occur carrying down with it nearly all the fat. When there is a distinct flocky precipitate no more acid should be added, as caseinogen is soluble though not very readily in excess. To precipitate the *whole* of the caseinogen the milk must be much diluted.

4. Filter off the precipitate. The filtrate should be clear; if it is not, either too little or too much acetic acid has been added; in this case add either a little more acetic acid or a little dilute sodium carbonate and filter again.

Boil a portion of the filtrate; a precipitate of **albumin** (with a little globulin) takes place. Filter, and to the filtrate

Apply Trommer's test (p. 180), a yellow or red precipitate will be obtained, showing the presence of a reducing sugar, **lactose**.

5. Carefully neutralise a portion of the filtrate from § 3 with sodium carbonate. A precipitate forms which does not dissolve in excess of alkali. This is calcium phosphate. Apply the nitric acid and ammonium molybdate test.

6. Boil some of the caseinogen precipitate § 3 with strong NaHO, cool, and shake with a little ether. Allow a drop of the ethereal solution to evaporate on a slide or on blotting-paper. A greasy spot remains.

7. **Action of Gastric Juice on Milk.** Neutralize with dilute Na_2CO_3 a little artificial gastric juice, filter and add 5 c.c. of the filtrate to 5 c.c. of fresh milk, place in the warm.

Observe at short intervals the condition of the milk; it will soon form a firm clot so that the test-tube can with safety be held upside down, later the clot shrinks and presses out a nearly clear fluid; the clot continues to shrink for some time.

The **rennet-ferment** in the extract has clotted the

milk, converting the caseinogen into **casein**, and this has carried with it the greater number of the fat globules.

If the amount of rennet-ferment contained in the extract is large the clotting may be almost instantaneous; in this case the experiment should be repeated, taking a smaller quantity of the extract and without warming¹. The extract is neutralised since (cp. § 3) excess of acid of itself precipitates caseinogen.

8. To the milk clotted by rennet-ferment add 5 c.c. HCl .4 p.c. and warm for an hour or so, the casein will be converted into peptone by the pepsin of the extract in the presence of acid.

¹ Some samples of commercial pepsin do not contain rennin. Commercial rennet may be used.

LESSON XXIII.

INTESTINE. BILE.

A. STRUCTURE OF INTESTINE.

1. Vertical sections of a cat's or dog's **small intestine** given out in paraffin (chromic acid ·2 p.c.; stained in bulk with hæmatoxylin)¹. The outer coats of the intestine have the same general characters as those of the stomach (Lesson XXII. A. § 1), except that there are no oblique muscular bundles. Observe in the mucous coat,

a. The projections of the mucous membrane, or **villi**, either extended and long, or contracted and short, with the surface thrown into folds. Note in the villi,

The epithelium, consisting of rather long **columnar cells**, each with a **hyaline border** more or less distinctly striated with vertical lines (the balsam is apt to render this indistinct), rather granular cell substance, and oval nucleus placed at about the lower third of the cell; the hyaline borders of the cells frequently appear to have coalesced into a narrow

¹ Or Flemming's fluid, cut in paraffin, stained on a cover-slip with Ehrlich-Biondi fluid.

highly refractive band, which may be traced over the whole villus.

The **mucous** or **goblet** cells, irregularly scattered among the former, sometimes abundant, sometimes scanty or absent; they have an upper ovoid portion which is transparent but has sharp outlines, and a lower basal granular portion containing the nucleus (cp. with the mucous cells Lesson XXI. § 4, and Lesson IX. § 1, b).

The connective tissue, forming the substance of the villus: this consists of a meshwork of fibres and membranous cells, for the most part hidden by the numerous leucocytes.

The 'lacteal radicle' may be visible in some of the villi as a central space bounded by a fine line formed by the epithelioid cells.

Unstriped muscular fibres as narrow bands running up the villus from the muscularis mucosæ.

b. The rather deep depressions of the mucous membrane, the **intestinal glands** or **glands of Lieberkühn**. Note that

The epithelium consists chiefly of cubical or short columnar cells; observe their gradation into the cells covering the villi, usually they have a small hyaline border similar to that of the columnar cells of the villi. Some goblet cells will be seen; (the number and appearance of the mucous cells vary in different animals).

There is usually a distinct basement membrane immediately beneath the epithelium, formed of connective-tissue cells very much flattened; the outlines of the cells are not seen in the section, but the nuclei are fairly conspicuous.

The lumina of the glands are small but usually distinct.

c. The adenoid tissue around the bases of the glands of Lieberkühn and between them and the muscularis mucosæ. This, unlike the corresponding tissue in the stomach (Lesson XXII. A. § 1, b), has a large number of leucocytes in its meshes.

d. The **lymph follicles**; they are round or oval masses of adenoid tissue crowded with leucocytes, lying immediately beneath the surface epithelium and stretching down into the submucous tissue. Villi are absent over them, and glands of Lieberkühn only occur over their peripheral portions. (The lymph follicles do not occur in every section.)

2. Transverse sections of the villi of the small intestine of dog. (Flemming's fluid, stained in bulk in hæmatoxylin.) Note, comparing with § 1, the cells with hyaline border and the goblet cells, the basement membrane, the connective tissue network with contained leucocytes, the capillaries a little below the basement membrane (these will not be obvious, if they are collapsed), the bundles of muscle-cells. In some sections the central lymphatic space will be seen, bounded by a sharp line probably showing one or more nuclei of its constituent cells.

3. Snip off a few villi from a fresh intestine, and tease in salt solution. Note especially the hyaline border of the columnar cells and its striation.

4. Vertical transverse sections of the **large intestine**. Observe

The longitudinal and circular muscular coats.

The mucous membrane, probably thrown into longitudinal ridges, the submucous tissue running up into the ridges.

The absence of villi.

The intestinal glands (glands of Lieberkühn); they are broader than in the small intestine, and have more connective tissue (chiefly adenoid) between them. The epithelium covering the free intestinal surface or the ridges between the glands consists of long columnar cells, in the glands the cells are shorter. The cells have usually a thin hyaline border. In some animals (*e.g.* dog) there are many distinct mucous cells.

5. Examine sections of a small intestine in which the blood vessels have been injected, and note the capillary network round the glands of Lieberkühn, and the small artery running up each villus and dividing into a capillary network just below the epithelium.

6. Section of frog's intestine after feeding with fat (osmic acid). The columnar cells contain numerous fat globules of various size, the hyaline border is free from fat.

7. Feed a frog with a small piece of bacon; on the next day¹ kill the frog, remove the stomach and intestine, pin the tube out on cork, cut it open, and gently wash with salt solution.

Note that the mucous membrane of the stomach has a yellowish semi-transparent look, whilst the mucous membrane

¹ The difference in the tint of the stomach and intestine is still more obvious if the frog be fed again after two days and killed on the subsequent day. The frog is fed by placing the piece of fat in the upper part of the œsophagus, the fat is then usually swallowed at once.

of the intestine is of an opaque white, this is more marked in the upper than in the lower part of the intestine ; the rectum is greyish and semi-transparent. Tease out a small piece of the opaque white mucous membrane in normal salt solution ; the epithelium cells are crowded with fat globules, scarcely anything but these being visible. Fat is absorbed by the cells of the small intestine, and is absorbed little or not at all by the cells of the stomach.

8. Pin out pieces of the intestine ; place some in 75 p.c. alcohol for an hour, and then in strong spirit ; place others in osmic acid 1 p.c. for half-an-hour, wash and place in 75 p.c. alcohol.

In sections of these pieces note that there are no villi and no proper glands of Lieberkühn. The mucous membrane is however thrown up into considerable folds. In the osmic acid specimens, the cells will probably be so full of deeply stained fat globules that little structure can be seen in them except the hyaline free border ; in the submucous connective tissue few or no fat globules are seen.

In the alcohol specimens the cell substance will be seen as a distinct sponge-work or network, the fat globules having been dissolved.

B. BILE.

1. Test the reaction of bile¹ with litmus paper. If fresh it is *slightly alkaline* or *neutral*.

2. To a small quantity add strong acetic acid drop by drop. A curdy precipitate of a mucoid body (mucin or nucleo-proteid or both), coloured with the bile-pigment will be thrown down (cp. Less. x. 6 ; XXII. C.). Since this body is not formed in the liver but in the glands and cells of the gall-bladder and duct, the longer

¹ Ox-gall or sheep's gall may be obtained from a butcher's.

the bile has been in the gall-bladder the greater the precipitate which will be obtained.

For the following tests (§§ 4, 5) it is best, although not necessary, to precipitate the mucoid body with acetic acid, to filter and use the filtrate; before filtering, the bile may be diluted four or five times with water.

The mucin and nucleo-proteid may also be removed by adding an excess of alcohol; the filtrate from this should be evaporated to dryness, and the residue dissolved in water.

3. *Gmelin's test for bile-pigment.* To a small quantity in a test-tube add drop by drop, nitric acid, yellow with nitrous acid, shaking after each drop; the yellowish green colour becomes first a dark green, then blue, then violet, then red, and finally a dirty yellow. The blue and violet colours are less obvious than the rest. Repeat the test in the following form; place a drop of bile on a porcelain slab, and place a drop of yellow nitric acid so that it runs into the drop of bile; where the fluids mingle, zones of colour, green, blue, violet, red, and yellow, from the bile to the acid, are seen.

4. *Pettenkofer's test for bile-acids.* To a little bile in a test-tube add one drop of a 10 p.c. solution of cane sugar (or a small particle of sugar) and shake. Add strong sulphuric acid to nearly the same amount as the bile taken, inclining the test-tube so that the acid settles at the bottom. Gently shake the test-tube from side to side, when the fluids have nearly mixed a deep purple colour is produced. If too much sugar is added the fluid will turn brown or black; if too little

sulphuric acid is added the proper temperature (about 70° C.) for the production of the colour will not be obtained.

As a control repeat the experiment with distilled water, adding the same quantity of sugar and sulphuric acid as before, and compare the brown colour so produced with the purple due to bile salts.

5. Repeat the experiment, using .1 p.c. furfural solution instead of cane sugar. Dilute with alcohol the purple solution obtained until the characteristic absorption bands of the products can be obtained with the spectroscope. A band is seen between *D* and *E*, and another near *F*.

6. Add a few drops of oleic acid to 10 c.c. of bile in a test-tube, shake well, and at once mount a drop and observe in it under the microscope the numerous fatty globules. Place the test-tube with the bile in a warm bath for an hour or so, then shake and mount a drop of the fluid; comparatively few fatty globules will be seen in it under the microscope. The oleic acid has combined with the base of the bile-salts to form a *soap*.

7. Place in separate test-tubes 10 c.c. of bile and a couple of drops of oleic acid (*a*); 10 c.c. of bile (*b*); 10 c.c. of water (*c*); to each add 2.5 c.c. of melted fresh butter¹, shake well, and place in the warm bath. The emulsion will last much longer in (*a*) than in (*b*); it will last much longer in (*b*) than in (*c*). *The emulsifying power of bile is slight; but in the presence of*

¹ If olive oil is used instead of melted butter, it will depend upon the amount of fatty acids contained in the olive oil whether any difference is observed in (*a*) and (*b*).

fatty acids it forms soaps (cp. § 5) which have a much greater emulsifying power.

8. Mount a few crystals of **cholesterin**¹ in water and examine them under a microscope, they consist of rhombic plates.

9. Irrigate the crystals with strong sulphuric acid; they turn red.

10. To a small quantity of chloroform in a test-tube add a little cholesterin and shake, the cholesterin will dissolve; add strong sulphuric acid and gently shake, the upper (chloroform) layer will turn bright red.

11. Digest a little fibrin in 10 c.c. of artificial gastric juice; when the fibrin is dissolved add drop by drop, bile which has been decolorized by filtering through animal charcoal; a precipitate will be formed containing acid albumin and bile acids. (If excess of bile is added the bile acids precipitated will be more or less completely dissolved.)

12. Add to the preceding 5 c.c. of .4 p.c. HCl and a few flocks of swollen fibrin; the fibrin will shrink and will be digested slowly or not at all. Bile, chiefly the taurocholate or its salt, delays the gastric digestion of proteids.

¹ Cholesterin may be prepared from gall stones (those which have a soapy feel) in the following manner. Powder the gall stones and add a small quantity of strong spirit (or absolute alcohol) and boil; filter hot, using a hot-water funnel; on cooling, cholesterin crystals will separate out.

DEMONSTRATIONS.

1. Vertical longitudinal section through the pylorus and beginning of the duodenum, of cat or dog. Note that the pyloric glands become shorter near the pylorus; at the beginning of the duodenum are Brunner's glands¹, forming a layer external to the muscularis mucosæ. In places a duct may be seen running down into the sub-mucous tissue, there it divides and subdivides, the end-tubes enlarging slightly, and forming alveoli; the cells resemble in general features the pyloric gland cells; the lumina are obvious.

2. Section through Peyer's patch. Note the collection of lymph follicles (cp. A. § 1, *d*).

3. Transverse section through Lieberkühn's glands of small intestine.

¹ Brunner's glands are massed close to the pylorus in Carnivora and in Rodents; in some other animals they are more scattered. The appearance of the gland-cells varies somewhat in different animals.

LESSON XXIV.

PANCREAS. PANCREATIC JUICE.

A. STRUCTURE OF PANCREAS.

1. Section of pancreas of frog¹ (osmic acid, cut frozen), mount in glycerine. Observe

(a) (l. p.), the lobules with their alveoli and ducts; the arrangement resembling in general features that of the salivary glands: the ducts, however, have a larger lumen, and cubical epithelium (cp. Lesson XXI. § 2).

(b) (h. p.). The cells contain a number of separate, spherical granules, on the inner or lumen side. How far these stretch towards the outer limit of the cells depends upon the state of digestion at the time the animal was killed, but there will probably be a more or less obvious outer non-granular zone. The outlines of the cells and nuclei, as usual after osmic acid fixation, are not very obvious; the nucleolus of each nucleus is generally conspicuous.

¹ In mammals the granules are smaller than in the frog; but the pancreas of a mammal may be taken.

2. Section of active pancreas of a dog¹ (alcohol); stain with picrocarmine or hæmatoxylin. The division of the cells into granular and non-granular zones will be seen; the inner zone however appears as a confusedly granular mass, and does not show the separate spherical granules of the osmic acid preparation (§ 1); the outer zone, containing the nucleus, is homogeneous, or nearly so, and stains more deeply than the inner zone.

3. Examine section of pancreas for the *inter-tubular* clumps. In mammals they form roundish clumps of small cells generally of polyhedral outline; they have no obvious granules, and stain very little with carmine or hæmatoxylin. In the frog the cells are elongated, and arranged in indistinct columns.

B. PANCREATIC JUICE.

1. *Preparation of pancreatic extract.* a. Mince finely a pancreas from a just killed animal, pound it well with clean sand and add about 100 vols. Na_2CO_3 .2 p.c. and a little thymol. Place it in the warm for some hours to a day, strain through muslin, filter through linen and then through filter paper.

b. Mince a perfectly fresh pancreas, grind it with sand or powdered glass, gradually adding glycerine containing $\frac{1}{10}$ th of a 1 p.c. sol. of Na_2CO_3 , taking about 10 c.c. of the fluid to each gram of pancreas. The extraction may be allowed to continue for not more than three days, then or earlier, strain or centrifugalise the fluid.

c. Mince a pancreas and pound it with sand, for each gram of gland-substance add 1 c.c. of acetic acid 1 p.c. and

¹ A mammal should be killed 5 to 8 hours after a full meal to obtain the active gland, and one 12 to 16 hours after a full meal for the resting gland. In the frog, the corresponding times are 8 to 10 hours, and 1 to 2 days; the frogs should be fed with worms; in unhealthy frogs and in those which have long fasted, the pancreas cells have usually a distinct outer non-granular zone.

mix thoroughly in the mortar for ten minutes ; add ten times its bulk of glycerine. In a day or two a little strong solution of sodic carbonate should be added to make the fluid slightly alkaline. When required for use add sodium carbonate as in *b*.

In the following commercial pancreatic extract may be used.

2. To about 5 c.c. of distilled water in a test-tube add a drop of oleic acid and shake, the fatty globules soon rise to the surface ; add 5 c.c. Na_2CO_3 1 p.c. ; a white precipitate of *soap* forms ; shake, the precipitate partially dissolves and more completely or wholly on boiling. Examine a drop of the fluid under the microscope, no fat globules will be seen.

3. Place in a warm bath two test-tubes, each containing 5 c.c. Na_2CO_3 1 p.c. ; melt a little fresh butter in a porcelain dish over a flame and with a warm pipette add an equal quantity (about 2.5 c.c.) of the melted butter to the fluid in each test-tube. To one of these (*a*) add a couple of drops of oleic acid. Shake the test-tubes and replace them in the warm bath, examining them from time to time ; the fine emulsion formed on shaking the fluids will last much longer in (*a*) than in (*b*). The emulsion in this case is much greater than with bile (cp. Less. XXIII. B, § 6).

4. Make observations on the amylolytic ferment of the pancreas similar to those made in Less. XXI. on the amylolytic ferment of saliva.

5. Test the proteolytic action of the pancreatic extract in a similar manner to that in which the action

of artificial gastric juice was tested (Less. XXII.) substituting 1 p.c. Na_2CO_3 for .2 p.c. HCl .

In test-tubes *A* and *D* the fibrin will be unaltered, in *C* it will be very slowly dissolved, in *B* it will be rapidly dissolved, hence sodium carbonate alone does not digest fibrin (*A*), trypsin alone digests it very slowly (*C*), trypsin in the presence of sodium carbonate dissolves it rapidly (*B*), the digestive power of trypsin being destroyed by boiling (*D*).

6. Let an active pancreatic extract act upon fibrin or white of egg in the warm for 24—36 hrs., thymol being added to prevent putrefaction. Boil the fluid and filter. Evaporate the filtrate to a small bulk on a water bath. Examine a drop microscopically for crystals of *tyrosin*. They consist of feathery masses of fine needles. Add to the remainder an excess of hot alcohol, and while hot filter off precipitated peptones. Concentrate the alcoholic filtrate and examine a drop microscopically for crystals of *leucin*. They consist of rounded clumps with radiating striation.

7. To 10 c.c. of extract *b* add 5 c.c. of an emulsion of oil of almonds and a little neutral litmus solution. Place in the warm. In a short time the litmus solution will be turned red. The fat-decomposing ferment of the pancreas has split up the neutral fat into fatty acid and glycerine.

DEMONSTRATIONS.

1. The appearance of the chyle in the lacteals of the mesentery of a rabbit a few hours after a meal.

2. The flow from the thoracic duct.

LESSON XXV.

THE LYMPHATIC SYSTEM.

A. LYMPHATIC GLANDS.

1. Vertical sections of a rabbit's **Peyer's patch** (ammonium bichromate 5 p.c.), stain with picrocarmine. Shake with water in a test-tube. Look at a section under a low power to see if the leucocytes are for the most part shaken out; if so mount it in glycerine. Observe

The group of lymphatic follicles, each follicle on one side is covered by the intestinal epithelium, and on the other extends into the sub-mucous coat.

The **adenoid tissue** of the follicle, continuous more or less distinctly with the neighbouring adenoid tissue, and resembling it except that the fibres are, as a rule, finer, and the meshes smaller.

Around parts of the follicle, narrow spaces between it and the surrounding tissue; these represent the **lymph-sinus** on the outside of the follicle.

2. Sections of a small lymphatic gland, including the hilus (one of the upper cervical glands of cat or dog; ammonium bichromate¹ 2 to 5 p.c.; cut frozen). Shake the sections with water in a test-tube to get rid of most of the leucocytes, stain with picrocarmine and mount in glycerine.

a. Observe under a low power

The connective-tissue of the **capsule** surrounding the gland and sending in

The trabeculæ, which divide the outer portion of the gland, the **cortex**, into compartments, the **alveoli**, and which then in the inner portion split up into bands, forming a network with rather narrow, elongated meshes, the **medulla** of the gland.

In the alveoli of the cortex the roundish masses of tissue crowded with leucocytes, the **follicles** of the cortex; in the intertrabecular spaces of the medulla the elongated masses of similar tissue, the **medullary cords**. Note that the follicles are continuous with the medullary cords.

Around the follicles and around the medullary cords and separating them from the trabeculæ the **lymph-channels** comparatively free from leucocytes.

¹ Good specimens are more certainly obtained by the following method. A cat or dog is killed (best by bleeding after chloroform has been given) and warm salt solution is injected into a carotid for a quarter to half-an-hour, the lymphatic glands of the neck are then cut out and placed in ammonium bichromate 5 p.c. for a few days, sections are cut with a freezing microtome and shaken. In sections so prepared the lymph channels are almost completely free from leucocytes and by careful and more prolonged shaking they may be removed very largely from the follicles and medullary cords.

b. Observe under a high power

The connective-tissue of the capsule and trabeculæ (in some animals, *e.g.* ox, this contains unstriated muscular fibres) continuous with

The reticulum of the lymph-channels.

Limiting the follicles and medullary cords may usually be seen a fine line with nuclei at intervals, indicating the flat cells bounding the lymph channel.

The adenoid tissue of the follicles and cords, with finer fibres and smaller meshes than that of the lymph channels; unless the section has been well shaken this will be largely hidden by leucocytes.

3. Section of lymphatic gland (Flemming's fluid), stain with safranin. Note that some of the follicles have a central round mass, the *germinal area*, staining more deeply at the periphery. They resemble in appearance the Malpighian corpuscles of the spleen; in them, leucocytes with dividing nuclei, deeply stained, may be indistinctly seen.

B. SPLEEN.

1. Piece of spleen of cat (ammonium bichromate 5 p.c.), cut at right angles to the long axis of the organ including rather more than half of the transverse surface. Note, with and without the aid of a lens, the round whitish Malpighian corpuscles. Prepare sections with the freezing microtome (cp. Less. v.). Stain some sections with picrocarmine and mount in glycerine, others with hæmatoxylin and eosin or picric acid and mount in balsam. Observe under a low power

Externally the broad fibrous sheath, the **capsule** sending in

Large and conspicuous **trabeculæ**; these run throughout the spleen, branching as they go into roundish bundles which are connected with other similar bundles, and so form an irregular trabecular network throughout the spleen: the bars of the network cut in all directions will be seen scattered about the section.

In many of the trabeculæ, largish central spaces, the **veins**, will be seen, devoid of any proper muscular and connective tissue coats; in the centre of the section large trabeculæ cut transversely or obliquely will probably be seen containing both arteries and veins; if the section passes through the point of entrance of the vessels these will be seen running towards the centre surrounded by tissue continuous with the capsule.

The **splenic pulp** occupying the spaces of the trabecular network; it resembles somewhat the follicular substance of the lymphatic glands but has a mottled appearance, in it will be seen roundish masses of tissue, the **Malpighian corpuscles** more deeply stained than the splenic pulp: when the corpuscle is cut transversely to the artery which it surrounds, the artery will be seen to be near one side (all the small arteries in the pulp are surrounded by a variable amount of adenoid tissue).

Examine the splenic pulp under a high power and note that the mottled appearance is due to the presence of red blood corpuscles scattered irregularly in it. (These are stained yellow with picric acid and orange with eosin.)

2. Thin section of a dog's spleen, washed out by injection¹. Stain as in § 1. Observe (h. p.)

There are no distinct lymph-channels.

The reticulum of the splenic pulp varies in appearance in different places; in places it appears as a network of cells having in various directions flange-like projections which taper off and join with the similar processes of neighbouring cells; elsewhere the cells may be nearly or wholly absent and a reticulum of fine fibres may be seen. Some leucocytes and red blood corpuscles not washed out of the reticulum will be present.

The reticulum of the Malpighian corpuscles resembles that of the follicles of lymphatic glands, in its meshes are many leucocytes, but no red blood corpuscles.

The small arteries, capillaries and veins of the pulp; the veins branch out from the trabeculæ and have sharp outlines with nuclei at intervals (they may usually be recognized in the dog by the spiral lines running round them).

The trabeculæ are chiefly composed of unstriated

¹ As soon as possible after the animal has been killed (best by bleeding under chloroform) all the branches of the cœliac artery except the splenic branches are tied and warm salt solution is injected into the artery until the spleen is quite pale; then 5 p.c. ammonium bichromate is injected until the spleen is yellow, the splenic veins are then ligatured, the spleen a little distended by further injection and the arteries tied. The spleen is removed to 5 p.c. ammonium bichromate; in two days it is cut in pieces and left in bichromate solution for a week or longer. The pieces are then placed in 30 p.c. alcohol, which is renewed until it is no longer coloured, sections may then be made (best with the freezing microtome) or the pieces may be kept in 75 p.c. alcohol.

muscle tissue (the amount of this varies in different animals, in some it is very small).

3. Section of spleen injected¹ with Berlin blue from the splenic artery under a low pressure. Mount in balsam. Note (l. p.)

The small arteries branching off to the Malpighian corpuscles; the artery penetrates the corpuscles eccentrically, and either divides into a number of capillaries which form a network in the Malpighian corpuscle, or passes on into the pulp, giving off a branch which divides into capillaries in the corpuscle.

Capillaries in the adenoid sheath of the arteries, but less numerous than in the Malpighian corpuscle.

Small arteries dividing into capillaries in the splenic pulp.

Small tufts of splenic pulp injected with blue at the ends of the capillaries of the pulp.

Irregular masses of injected splenic pulp outside the Malpighian corpuscles and adenoid tissue of the arteries where the capillaries open out into the pulp.

¹ A dog is perhaps the best for injection, but a cat or a rat answer the purpose very well. In a dog the individual arteries and veins which run to the spleen are large enough to be easily injected separately, and since the fluid (especially with an arterial injection) does not readily spread out beyond the section of the spleen directly supplied by the vessel, a number of injections may be made in the same animal. The whole spleen should be washed out first from the celiac artery. The best injection material is a .2 p.c. solution of nitrate of silver, after the injection pieces of the spleen are put in 75 p.c. alcohol for a day or two, then cut with the freezing microtome and exposed to the light. The disappearance of the epithelium of the capillaries and small veins of the splenic pulp show in the clearest manner the opening of these into the spaces of the pulp.

If too great a pressure has been used in injecting, instead of the tufts of injected pulp at the end of the capillaries, irregular areas of the pulp or the whole of it will be permeated with the Berlin blue; the Malpighian corpuscles and the adenoid tissue of the arteries will be free from injection unless the pressure used in injecting has been very great.

C. SEROUS SPACES. LYMPHATIC CAPILLARIES.

1. **Epithelioid cells of serous membranes.**

Behead a pithed frog, squeeze the body upwards to remove as much blood as possible. Pin out the lower coil of the intestine with the mesentery (cp. Lesson XVIII. § 9); wash it with .75 p.c. sodium sulphate; add a drop or two of .2 p.c. silver nitrate, in 5 minutes wash with sodium sulphate. Cut away the intestine, expose the mesentery to light in distilled water. When the outlines of the surface cells show (about $\frac{1}{2}$ hour), place the mesentery on a slide, drain off the water, stretch, drop on it 95 p.c. alcohol to fix it, place in alcohol for 5 minutes. Mount in balsam.

Note the epithelioid cells covering the mesentery. They are large and flat; and for the most part polyhedral.

2. **Epithelioid cells of lymphatic¹ spaces.**

Follow the directions given in Lesson xv. § 9, up to the point at which the peritoneum is seen stretching from the lateral edge of the kidney to the abdominal wall. Keeping the kidney lifted, so that the peritoneum

¹ The dissection is simpler in the male frog, cp. Footnote p. 150.

is kept taut, let a drop or two of .75 p.c. sodium sulphate run over both sides of the membrane, then, after sopping up the fluid with a small piece of sponge, repeat the process, but using .2 p.c. silver nitrate. In 3 to 5 minutes cut through the abdominal attachment of the peritoneum, transfer it to sodium sulphate .75 p.c. (or to water), cut off the kidney. Cut the peritoneum into two unequal pieces, so that they may be recognized, and expose to light in water, one with the abdominal and the other with the lymphatic surface uppermost, and treat in the manner given in § 1. Observe on the external side, (a) the lymphatic epithelium, consisting of large flat cells with very sinuous outline, (b) the *stomata*, each has a small opening between three or four small cells, more deeply stained than the rest; the outlines of the surrounding epithelioid cells radiate from them.

On the peritoneal side, cells less sinuous in outline than the lymphatic epithelium, but more irregular than the cells covering the mesentery; stomata will also be seen. (The epithelium of both sides can probably be seen in the same preparation, on focussing.)

3. Treat with .2 p.c. nitrate of silver the peritoneal surface of the diaphragm of a guinea-pig and mount, peritoneal surface uppermost. Observe

The tendon bundles of the diaphragm arranged in two layers. The spaces between the bundles mark for the most part the course of the lymphatic capillaries of the tendon (cp. § 4).

Superficial to the tendon bundles the epithelium of the peritoneum consisting of flat polygonal cells. These are larger over the tendinous bundles than over the intervening spaces. Stomata similar to those of § 2 may be seen, situated over and communicating with the inter-tendinous spaces. Pseudo-

stomata, irregular patches of staining substance at the junctions of the cells, are frequent.

4. In a guinea-pig or rabbit, brush firmly the pleural surface of the diaphragm with a camel-hair brush to remove the surface epithelium and treat with nitrate of silver. Mount with the pleural surface uppermost. Note

The *small lymphatic vessels*, running a little above the tendinous bundles and lined with somewhat irregular spindle-shaped epithelium. In places the curved outline of a *valve* may be seen.

The superficial lymphatic capillaries with their characteristic sinuous epithelium continuous with the lymphatic capillaries of the inter-tendinous spaces.

The origin of the lymphatics. This is best seen in specimens deeply stained with nitrate of silver, so as to produce the so-called negative image. Note the clear branched spaces, whose sinuous outline resembles that of an epithelium cell of a lymphatic capillary. The clear spaces, cavities containing unstained connective-tissue corpuscles, stand out in strong contrast with the surrounding stained matrix. It may be seen that these spaces here and there apparently form the beginning of a lymphatic capillary.

DEMONSTRATIONS.

1. Section of Peyer's patch with blood vessels injected. Note in the follicles the radial arrangement of the capillaries.

2. Section of Peyer's patch with the lymphatic system injected. Note that the injected material envelops to a greater or less extent each follicle; it occupies the lymph sinus (cp. A, § 1), and does not penetrate into the interior of the follicle.

3. Section of small lymphatic gland with blood vessels injected. The arteries enter at the hilus surrounded by connective tissue and branch in the trabeculæ of the gland; from the smaller of these branches fine arteries run to the follicles and medullary cords and form in them a capillary network; no capillaries occur in the lymph-channels. The veins have a distribution similar to that of the arteries.

4. Section of spleen injected with Berlin blue from the splenic vein under a low pressure. Observe, the veins in the trabeculæ filled with injection material; the veins of the pulp more or less distinctly branching out from the trabeculæ; the irregular masses of injected pulp at the ends of the veins.

5. Transverse section of thoracic duct.

6. Specimen to show lymphatics of pleural surface of the diaphragm of guinea-pig (cp. C, § 4).

7. Specimen to show injected lymphatics of tendon of diaphragm. (2 p.c. solution of Berlin blue placed in abdominal cavity of guinea-pig or rabbit under chloroform; animal killed in an hour or two.)

LESSON XXVI.

STRUCTURE OF LIVER. GLYCOGEN.

A. STRUCTURE OF LIVER.

1. Sections of frog's liver (osmic acid). Mount in dilute glycerine. Observe with a low power that the gland apart from the ducts and ductules consists of **anastomosing tubes** between which the blood capillaries run. Observe (h. p.)

The **tubes**. In transverse sections they are seen to consist of four to six cells, each containing a large nucleus in its outer portion.

The **bile capillaries**; these are the lumina of the tubes; in longitudinal sections of the tubes the bile capillaries are seen to take a zigzag course between the inner ends of the cells.

The **cell granules** arranged, according to the condition of the liver, either throughout the cells or around the lumen.

The **fat globules**, stained black with osmic acid; they vary greatly both in number and position according to the condition of the liver.

The **glycogen** content of the cells, indicated roughly by the extent of the non-granular outer zone. Mount a section in aqueous solution of iodine; the parts of the cell containing glycogen will stain a deep brown-red (cp. B, § 2, *a*).

2. Section of mammalian liver, preferably that of a pig made parallel to the surface of the liver. (Müller's fluid or potassium bichromate 2 p.c.) Stain with hæmatoxylin.

a. Observe with a low power

The division into **lobules**; the connective tissue between the lobules is greater in the pig than in the dog or rabbit.

In the centre of most of the lobules the very thin-walled **hepatic** or **intralobular veinlet**; those in which it is not seen have been cut through near the outer end of the lobule.

Between the lobules the thin-walled **portal** or **interlobular veinlets**, some of them of considerable size; the **branches** of the **hepatic artery**, small but with comparatively thick walls; and the small **bile-ducts**, with cubical or columnar epithelium and distinct lumina. The three vessels run together.

The **hepatic cells** radiating more or less obviously from the hepatic veinlet.

b. Observe with a high power, the polygonal hepatic cells with granular cell substance and one or two spheroidal nuclei, and the epithelium of the bile-ducts.

3. Section of liver, the blood vessels of which have been injected with Berlin blue or with carmine-gelatine.

Mount in balsam. Observe, comparing with the uninjected specimens,

The hepatic veinlet, seen according to the plane in which the lobule is cut, either as a more or less circular section, or as a short veinlet passing from the centre of the lobule to the sub-lobular vein.

The portal veinlet running on the outside of the lobule, and giving branches to more than one lobule.

The radial capillary network between the portal and hepatic veinlets united by numerous cross branches; commonly there is only one row of cells between the radial capillaries.

4. Section of rabbit's liver containing glycogen (alcohol, cut frozen), cp. B, § 1. Place in a drop of iodine solution, and mount in glycerine containing a little iodine, observe the red-brown stain of glycogen in the cells.

5. Scrape a small portion of fresh liver, and observe the pale, granular, hepatic cells; often containing fat globules.

6. *Test for iron.* (a) Place sections of liver¹ in a mixture of equal parts of 2 p.c. hydrochloric acid, and 2 p.c. potassic ferrocyanide, warm for a few minutes, wash and mount in balsam. Particles containing iron or certain simple organic compounds of iron will be stained deep blue. (Prussian blue reaction.) (b) Mount the section in a strong solution of sulphide of ammonium, the particles containing iron will be stained black (ferrous sulphide reaction).

¹ In a chloroformed animal inject diamine of toluylene into a vein, in 4 or 5 hours cut out the liver and preserve in alcohol. Iron containing particles will be present in the centre of the cell-columns of the liver.

B. GLYCOGEN.

1. Give a rabbit a full meal¹ and about six hours afterwards decapitate it, cut out the liver as rapidly as possible, disregarding the bleeding. Remove the gall-bladder and cut up one half of the liver into small pieces and throw them straightway into about 200 times their bulk of water which is already boiling. In two or three minutes, when the pieces in the boiling water are all thoroughly coagulated, and the ferment has been destroyed, remove them and pound them in a mortar into a paste with sand; mix this paste with the water previously used, just acidulate with dilute acetic acid (to ensure the complete coagulation of albumin) and boil for a few minutes. Let it stand till the coagulated proteids have settled, and filter the milky fluid through a coarse filter. The sediment may be squeezed in linen, and the expressed fluid thrown on the filter. The opalescent filtrate is a crude infusion of glycogen. If it contains *much* proteid material, it should be carefully neutralized, boiled again, and filtered.

Place the other half in the warm for some hours, keeping it moist, then treat as the first half was treated. If acid, neutralize with sodium carbonate and filter.

2. Extract of fresh liver (cp. § 1). *a.* To a few c.c. of the fluid add a drop or two of iodine solution. A port-wine colour will result which will rapidly disappear if much glycogen is present, if so add more iodine until the colour is permanent. Warm gently; the colour will disappear, but will return on cooling (unless much proteid matter be present).

b. Test 5 c.c. for sugar with Trommer's test (cp. Less. XXI. B, 3); a small quantity only will be found.

c. Add to 10 c.c. (neutralizing if acid) a little

¹ The rabbit may be fed on bran and carrots.

saliva, or pancreatic extract, and place it in the warm chamber; the opalescence will disappear, and the fluid become transparent.

To 5 c.c. of this add iodine as before; the port-wine colour will not appear, showing that glycogen is no longer present.

Test the other 5 c.c. for sugar; much more than before will be found.

d. Add to a little of the fluid twice its vol. of 90 p.c. alcohol, so as to have not less than 60 p.c. in the whole; shake, let stand for 5-10 min., glycogen is precipitated. Filter. Test the filtrate with iodine, only a faint port-wine colour is obtained.

3. Extract of liver prepared some hours after death (cp. § 1). Examine in the manner given in § 2, and compare the results.

a. The decoction will be clearer, more transparent, and less milky.

b. It will give less port-wine colour with iodine.

c. It will contain sugar (dextrose) in abundance.

d. It will give less precipitate with 60 p.c. of alcohol.

LESSON XXVII.

THE LUNGS. MECHANICS OF RESPIRATION.

1. Transverse section of the posterior part of a mammalian trachea (chromic acid ·2 p.c., hæmatoxylin), taken so as to include the ends of a tracheal ring, and the membranous part between them. Observe,

Externally the fibrous coat of loose connective tissue, becoming denser internally, and having imbedded in it the two ends of the ring of cartilage.

Stretching between and outside the ends of the cartilage, a **transverse band** of unstriped **muscular fibres**; there may also be visible outside this the cut ends of muscular fibres running longitudinally.

The **submucous coat** continuous with the fibrous coat, but of finer fibres. In this coat the small **glands** consisting chiefly of mucous cells, though some albuminous cells are usually present; the ducts are not very evident, but they may be seen running towards the surface, and occasionally opening on it.

The **mucous coat**, consisting from without inwards of (a) **longitudinal elastic fibres**, becoming finer towards the basement membrane (probably the network

formed by the elastic fibres will not be seen; some adenoid tissue may be present amongst the inner fibres), (b) the basement membrane, and (c) the epithelium, made up of **columnar ciliated cells**, and of two or three rows of small cells between the bases of the ciliated cells; some mucous cells also will probably be seen.

2. Longitudinal vertical section of trachea, taken through the tracheal rings. Compare it with the transverse section, noting especially the elastic fibres of the mucous coat.

3. Piece of mammalian lung taken from the more central portion (distended with, and hardened in, chromic acid '2 p.c.). Pass through alcohols down to 30 p.c. Place in acid hæmatoxylin for an hour or two. Imbed in paraffin (Lesson v. § 5) and cut sections. Observe

The **larger bronchia** (if present in the section) resemble in general features sections of the trachea except that the cartilages are irregular in form, and are irregularly scattered throughout the fibrous coat, the **circular muscular fibres** form a ring at the base of the mucous coat.

The **smaller bronchia** have no cartilage, the epithelium consists of a single layer of ciliated cells, elastic and muscular coats are present.

The **alveoli** having distinct outlines, partly due to blood vessels, and partly to elastic fibres on the walls, the nuclei of some of the epithelioid cells are seen, here and there the wall of the alveolus will be seen flat, it appears then as a thin nucleated membrane, the cell

outlines being invisible or barely visible (cp. Demonst. 3).

4. Section of mammalian lung, the blood vessels of which have been injected; mount in balsam.

Observe the close-set capillary network over the alveolus, and the artery forming a circle round the mouth of each alveolus.

5. Cut out a lung from a recently killed newt, open it longitudinally, divide into two parts and spread them out, inner surface uppermost, on separate slides, being careful not to rub off the epithelium.

a. Treat with silver nitrate as in Lesson XVIII. § 2, but do not transfer from clove oil to balsam until the outlines of the surface cells are visible.

b. Make a moist film preparation and fix with alcohol. Stain, but not very deeply, with hæmatoxylin and eosin.

Observe—The nuclei of the surface cells of the lung, occurring in groups of two to six. The outline of the cells (silver preparation). The close capillary network occupying almost all the space between the nuclear groups. The blood-corpuscles in the capillaries (alcohol specimen), stained orange with eosin; focus up and down and note how near the surface the corpuscles are.

The capillary network may be made obvious and the outlines of its constituent cells shown, by injecting the vessels with silver nitrate (cp. Lesson XVIII. § 5).

6. Distend the lungs of a pithed newt (or frog) with 30 p.c. alcohol; leave the lung in this fluid for two days, wash, place for a day in Ranvier's picrocarmine; wash, place in dilute glycerine. Scrape the inner surface, and tease the scrapings in dilute glycerine. Observe

The isolated lining cells of the lung, consisting of a thin hyaline ground plate, having near one border a nucleus with a little granular cell substance around it.

DEMONSTRATIONS.

1. Inner surface of frog's lung (distended and fixed with alcohol). Observe with the unaided eye, and with a lens, (a) the large central space, (b) the somewhat short primary septa running inwards from the wall of the lung, and forming a number of polygonal chambers open towards the central space, (c) short secondary septa, projecting into the chambers from the primary septa.

2. Section of newt's lung (distended with Flemming's fluid; picrocarmine). Note the epithelium cells, the thicker part between the capillaries containing the nucleus, the thin expansion of the cell lying over an adjoining capillary.

3. Section of mammalian lung treated with nitrate of silver¹ to show the epithelium cells of the alveoli.

4. The circulation in the lung of toad or frog².

¹ Expose the lungs of a decapitated rat. Inject into the trachea a mixture containing .25 p.c. silver nitrate, and .5 p.c. osmic acid; force the fluid repeatedly in and out, so as to remove the air as far as possible from the alveoli. In 15 minutes cut up the lungs, wash with water; place a piece in gum, cut it frozen, expose the sections to light for $\frac{1}{2}$ hour; mount in balsam. The other pieces may be kept in 70 p.c. alcohol.

² If the lungs collapse, and they usually do in a frog, take a cannula with two necks, about 4 mm. apart, and with a small hole in the glass between the necks. Slip over the end of the cannula a piece of the rectum of a frog, and tie it round each neck. Open widely the mouth of frog (brain destroyed, and curarised), put the cannula into the glottis, slightly inflate the lungs by pinching the tubing attached to the cannula, insert into the tubing a glass rod which fits it. The degree of distension of the lung can then be varied at will by pushing the rod in and out. The lung should not be much distended. A special stand is desirable.

5. Tracing on the revolving drum of the movements of the column of air in respiration.

6. The movements of the diaphragm.

7. Apnœa.

8. Slight dyspnœa.

9. The action of the respiratory muscles in dyspnœa.

10. The collapse of the lungs on puncturing the thorax, and the consequent futility of the respiratory movements.

11. The action of the phrenic nerve.

12. The action of the vagus and the superior laryngeal nerves.

LESSON XXVIII.

THE COLOUR OF BLOOD. RESPIRATION.

1. Pour a little defibrinated blood¹ into several test-tubes (*a*)—(*d*).

a. Keep for comparison with the rest.

b. Add an equal volume of water and warm to about 50° C.

c. Add a few drops of ether or chloroform and shake.

d. Add a little bile or solution of bile salts and shake.

The blood in (*b*) (*c*) (*d*) will be **laky**, *i.e.* comparatively transparent, owing to the hæmoglobin of the corpuscles having been dissolved in the fluid; compare the colour with that of (*a*), compare also the transparency of (*a*) (*c*) by placing a drop of each on a glass slide and attempting to read type through it.

2. Prepare **crystals of hæmoglobin** as follows:

a. Cut off the head of a rat, receive a drop of the blood on a slide, add a drop of distilled water, mix,

¹ Obtain blood from the butcher's and whip it.

cover with a cover-slip. Crystals usually form in a few minutes.

b. Cut off the head of a rat or guinea-pig. Collect the blood in a glass beaker, and defibrinate it; pour the defibrinated blood into a crucible surrounded by a mixture of ice and salt. Leave it till it is frozen. Then remove it from the ice and salt, so that it may thaw. By this means the blood-corpuscles will be broken up, and the blood will become laky (cp. § 1). If the blood does not become thoroughly laky it should be frozen and thawed again.

Place the laky blood on one side in a cool place (it is best to surround it with ice) for a day. A sediment will then have formed consisting partly of hæmoglobin crystals and partly of broken-up corpuscles.

c. To defibrinated dog's blood add ether gradually, shaking continuously until the blood becomes laky (the volume of ether required is about $\frac{1}{16}$ the vol. of the blood taken), place it then in an ice-chamber until the next day. The blood treated thus often yields crystals as soon as it is cooled.

d. Sometimes defibrinated guinea-pig's blood yields crystals when a drop of it is simply mounted with a drop of chloroform; usually crystals may be obtained by freezing the blood, mounting a small portion, and warming gently over a flame for about half a minute and then cooling; as the blood cools crystals separate out.

Examine (h.p.) the slides prepared in *a* and *d* and a little of the sediment formed in *b* and *c*. Note

The **crystals of hæmoglobin** (oxyhæmoglobin); those of the rat and dog are thin rhombic prisms; those of the guinea-pig are apparently tetrahedra but in reality belong also to the rhombic system. Look for a clump of crystals to observe better their bright red colour.

The decolorized red blood corpuscles seen as pale rings mixed up with a good deal of granular débris.

3. Let blood (a large quantity is best) clot in a beaker, leave it for a day, then pour off the serum, mince the clot and shake the fragments gently with an equal volume of cold water, place a piece of muslin over the beaker and pour off the fluid; repeat this two or three times, then treat the residue with about three times its volume of water (best at temperature of about 40°C.), squeezing the pieces; filter through a coarse filter. A crude solution of hæmoglobin is thus obtained.

4. Arrange a spectroscope so that the spectrum of a flame is distinctly seen. Hold in the flame a wire having a few crystals of common salt upon it and observe the bright yellow sodium line (D). Bring then the micrometer wire of the eye-piece exactly over it and read off on the vernier the position of the telescope. In the subsequent observations when the telescope is brought into the position read off, the micrometer wire will give the position of the D line.

If the spectroscope is furnished with a scale, shift the scale so that the sodium line is at 58.9 of the scale and clamp the spectroscope tubes.

The numbers on the scale indicate wave-lengths in hundred-thousandths of a millimetre, so that each division corresponds to a difference of a hundred-thousandth of a millimetre, and each tenth of a division corresponds to a millionth of a millimetre, in wave-length. The wave-length λ of the line D is 589 millionths of a millimetre, so that when this line is placed at 58.9 of the scale, the wave-lengths of the parts of the spectrum can be read off on the scale.

The spectra described below should be carefully drawn on a blank scale like that of the spectroscope, the position of Fraunhofer's lines B , C , D , E , F being filled in from the following table of the wave-lengths of these lines expressed

(roughly) in millionths of a millimetre, $B=687$, $C=657$, $D=589$, $E=527$, $F=486$. If practicable these lines should be observed in the solar spectrum.

Introduce between the flame and the spectroscope a much diluted solution of **oxyhæmoglobin**. Note

The two absorption bands, both between the lines D and E ; the one (α) near D being narrower and darker than the one (β) near E (if the solution is very dilute, (α) may be the only band seen).

The middle of (α) is about λ 578, that of (β) about λ 540. The mid-lines of the bands vary somewhat with the strength of the solution.

5. Increase gradually the strength of the solution.

The spectrum is more and more cut off, both at the blue and at the red end, but especially at the former. The absorption bands are both blacker and broader.

As the solution becomes stronger the two bands run together, the ends of the spectrum also suffering absorption, so that light passes through only in a space in the green and a broader space in the red.

With a still stronger solution the green light also is absorbed, and only the red is visible, and this at last disappears.

6. Reduce the oxyhæmoglobin solution by adding a few drops of ammonium sulphide solution and warming gently. Or reduce with freshly-prepared Stokes' fluid in the cold; this consists of ferrous sulphate rendered alkaline with ammonia, sufficient tartaric acid having been previously added to prevent precipitation.

Compare the claret colour of the **reduced hæmoglobin** solution with the bright scarlet of the original solution.

Examine with the spectroscope. There is a single broad band, occupying a position intermediate between those of the two oxyhæmoglobin bands which have disappeared. This single band is much less dark than either of the two bands produced by the same quantity of oxyhæmoglobin.

The band is not quite intermediate ; its mid-line (about λ 565) lies nearer *D* than *E*.

With stronger solutions less of the blue of the spectrum and more of the red is absorbed than with a solution of oxyhæmoglobin.

7. Shake well the reduced solution, pour it two or three times from one vessel into another so as to expose it thoroughly to air ; and examine it at once. The bright scarlet colour will be restored ; and the oxyhæmoglobin spectrum will reappear.

If allowed to remain at rest, reduction, from excess of reducing reagent present, may soon return.

8. Examine the spectrum of blood-crystals either with the microspectroscope or by placing a thick layer of crystals on a glass slide before the larger spectroscope. The spectrum of oxyhæmoglobin is seen.

9. Pass **carbonic oxide** through an oxyhæmoglobin solution for fifteen to thirty minutes.

Note the peculiar bluish tinge acquired. Examine the spectrum ; two bands are seen like those of oxyhæmoglobin, but both placed more towards the blue end. In the absence of a wave-length spectroscope, oxyhæmoglobin and CO-hæmoglobin may be compared as follows. Place some of the oxyhæmoglobin solution before the spectroscope, bring the micrometer wire to

the middle of one of the bands, and fix the telescope in position. Replace the oxyhæmoglobin solution by the carbonic oxide hæmoglobin solution and examine; the middle of the band will now be to the blue side of the wire.

The middle of (α) is about λ 572, of (β) about λ 535.

Treat the carbonic oxide hæmoglobin with either of the reducing agents used above. Reduction will not take place.

10. Add to a moderately strong solution of oxyhæmoglobin a few drops of a strong solution of potassium ferricyanide, warm gently, then allow to stand for a few minutes. Examine with the spectroscope, the oxyhæmoglobin bands will have disappeared and an absorption band in the red characteristic of *methæmoglobin* will be seen. The middle of the band is about λ 630. There is large absorption of both ends, particularly of the violet end, of the spectrum.

11. Mix defibrinated blood into a thin paste with solid potassium carbonate and evaporate the mixture to dryness on a water bath. Extract the powdered residue with boiling alcohol and filter, a solution of *hæmatin in alkaline alcohol* is obtained. Examine with the spectroscope, one absorption band just on the red side of the *D* line will be seen, and there is large absorption of the violet end of the spectrum. The middle of the band is about λ 600.

Reduce some of the solution with, *e.g.* Stokes' fluid (cp. § 6), the band is replaced by two situated in the green, the one nearest *D* (λ 560) being very dark and sharply defined, characteristic of *reduced hæmatin* or *hæmochromogen*.

Acidulate some of the alkaline solution of hæmatin with H_2SO_4 and observe the spectrum of *hæmatin in acid alcohol*. One absorption band will be seen in the red (λ 645) somewhat like the band of methæmoglobin (cp. § 10).

12. Take a few c.c. of a solution of crystals of hæmoglobin.

Boil; the proteid constituent will be coagulated.

Add drop by drop HCl 1 p.c., the hæmoglobin will be split up and the proteid constituent precipitated; add an equal bulk of ether and shake, the colouring matter (hæmatin) will be largely dissolved in the acid-ether; with a pipette remove the lower stratum of fluid, add a few drops more acid and place at about 40° C., the proteid precipitate will be converted into acid-albumin and dissolved; neutralize, it will be precipitated and may be examined for the ordinary characters of acid-albumin.

13. Place a drop of blood on a glass slide, and by *gently* warming evaporate it to dryness: add to it a grain of salt, and thoroughly mix it with the blood, rubbing the whole to a fine powder. Cover with a cover-slip, and let a little *glacial* acetic acid run under it. Warm the slide, not too rapidly, over a flame till bubbles appear under the cover-slip; then let it cool, and examine under a microscope with a high power. A large number of **crystals** of **hæmin** as brown-red rhombic prisms will be seen.

DEMONSTRATIONS.

1. Quantitative determination of the hæmoglobin in blood.
2. The gases of venous and arterial blood.
3. The respiratory function of the vagus and the superior laryngeal nerves.
4. The action of the respiratory centre.
5. The phenomena of asphyxia.

LESSON XXIX.

STRUCTURE OF THE KIDNEY.

1. **Dissection of sheep's kidney.** Cut the kidney in half longitudinally, note the **ureter** expanding into the **pelvis** and then into several tubes, the **calices**, into which the pyramids project.

On the cut surface of the kidney note internally the pale **medulla** formed by the pyramids of Malpighi; externally the **cortex**, a brownish-red zone, having thin pale radial stripes (cp. § 2, *a*) which do not quite reach the surface; and between the medulla and cortex the **intermediate layer**, forming a dark red zone not very sharply defined, especially on the cortex side, and having pale stripes continuous with those seen in the cortex running radially through it from the medulla.

Turning back to the ureter, note the connection of its outer connective tissue coat with the fibrous coat of the kidney, follow the renal artery and vein running into the kidney in the connective tissue outside the ureter and pelvis; both artery and vein divide into several branches which enter the substance of the kidney, between the ends of the calices, at the bases of the pyramids; tracing them outwards they will be seen to run to the outer portion of the intermediate

layer and there to branch, their branches arching through the kidney substance and so forming a network (more complete in the veins than in the arteries) stretching through the kidney substance in the curved surface of the outer part of the intermediate layer.

2. Radial section of mammalian kidney extending from the outer surface to the summit of a papilla (ammonium bichromate 5 p.c.). Stain with picrocarmine.

a. Observe under a low power

The **medulla**, with its straight tubes; some of the numerous divisions of these as they run outwards may be seen.

The **intermediate layer**: the straight tubes of the Malpighian pyramids separate into bundles the **medullary rays** (pyramids of Ferrein); between the bundles are seen numerous blood vessels and some tubes of Henle (cp. § 2, *b*).

The **cortex**: the medullary rays are seen to run out nearly to the free surface, between these are convoluted tubes and capsules with their glomeruli arranged in double rows between each two pyramids (the symmetrical arrangement of the medullary rays and intervening convoluted tubes may not be obvious if the section is cut obliquely). In the outer part of the cortex convoluted tubes only are seen.

b. Observe under a high power

a. In the medulla:—

The epithelium of the **straight tubes**; in the smaller tubes, collecting tubes, this is composed of short columnar or cubical cells with spherical or ovoid

nuclei; in the larger tubes, outflow tubes, it is composed of longer columnar cells with ovoid nuclei; the lumina, distinct throughout, become larger as the tubes increase in size.

β. In the intermediate layer:—

The continuation of the straight tubes outwards in the medullary rays.

The loops of Henle, only seen occasionally, chiefly in the medullary rays, they run down also a variable distance into the medulla.

The **ascending limbs** of the loops; these will probably be deeply stained, they vary in size in different parts of their course and are composed of cells sometimes imbricated, with striated outer portions and containing oval nuclei; the lumen is small.

The **descending limbs** of the loops; these are much narrower, with transparent flattened epithelium, the nuclei of which project into the lumen, sometimes alternately on the two sides, and thus the tube, except for its basement membrane, simulates a blood capillary.

The change in character of the epithelium may take place either in the ascending or in the descending limb of the loop.

The numerous blood vessels between the medullary rays (cp. § 6); in the outer part of the layer, rather large arteries and veins cut transversely or obliquely will be seen (cp. § 1).

γ. In the cortex:—

The **capsules**, with the nuclei of their epithelium.

The **glomerulus** in each capsule (cp. § 6) and the nuclei of its capillaries.

The narrow neck of the capsule; this will be obvious in those capsules only in which the section has passed longitudinally through the neck.

The coiled course of the **convoluted tubes**; the outlines of the individual cells may or may not be distinct, each has a spherical nucleus and is striated in its outer portion. Sometimes the lumen is large, sometimes it can scarcely be made out.

The continuation outwards in the medullary rays of the ascending loop of Henle.

In the outer half of the cortex are deeply stained short tubules running out from the rays and sometimes seen to be continuous with the ascending loops of Henle; their cells resemble those of the ascending loop except that they are of unequal size, thus giving a very zigzag outline to the tubule; this is the '*irregular*' portion of the urinary tubule.

In the medullary rays will also be seen one or two rather large tubes with conspicuous spherical nuclei, these are the so-called *spiral tubules*. Note also in the rays the smaller straight (collecting) tubes.

A basement membrane may be made out in all portions of the urinary tubule.

3. Section at right angles to the medullary rays through the deeper part of the cortex. Observe the medullary rays surrounded by convoluted tubes.

4. Tease a small piece of the cortex of a kidney which has been kept in 5 p.c. neutral ammonium chromate for 3 to 6 days.

Observe the cells of the convoluted tubes, isolated or in groups, showing a very distinct striated outer portion; in some cells the outer part may appear as a brush of 'rods.'

5. Cut freehand a thin section of the inner part of the

cortex of a fresh kidney, tease it out in normal salt solution and observe the appearance of the fresh cells in the isolated bits of tubules.

6. Radial section of kidney which has been injected from the renal artery. Mount in balsam. Observe

The large arteries and veins in the outer part of the intermediate layer.

The small arteries and veins (**arteriæ et venæ rectæ**) given off from these, running down between the medullary rays into the medulla; they break up almost immediately into a brush of capillaries which enter the medulla and form a network throughout it; the meshes are elongated in the direction of the tubes, especially near the summit of the papilla.

The **interlobular arteries** and **veins** running from the larger vessels outwards in the cortex between the medullary rays; the arteries give off on all sides (two rows will probably be seen in the section) small afferent arteries, one to each capsule, where it breaks up into capillaries to form the glomerulus.

The small efferent vein issuing from each capsule and breaking up into capillaries which form a network in the cortex; the veins from the innermost capsules break up into a brush of capillaries similar to that formed by the **arteriæ rectæ** and run towards the medulla.

The small veins running from the capillary network of the cortex to the interlobular veins.

Here and there the small artery running to a glomerulus may be seen to send a branch direct to the capillary network of the cortex; similar direct branches will also be seen in the outer part of the cortex running from the ends of the interlobular arteries.

Small veins at the periphery of the cortex (*venæ stellatæ*) also arising from the capillaries of the cortex.

7. Vertical section of a rabbit's or dog's **bladder** (distended with and hardened in ammonium bichromate 2 p.c.). Stain with hæmatoxylin. Observe

The thin external fibrous coat.

The muscular coat consisting of an outer generally speaking longitudinal layer, and an inner generally speaking circular layer; inside this may also be seen fibres running in various directions, chiefly longitudinally.

The sub-mucous coat of connective tissue.

The mucous coat of connective tissue, rather finer but continuous with that of the sub-mucous coat; and of stratified epithelium, this consists of an inner layer (next to the cavity of the bladder) of a single row of roughly cubical or flattened cells, a median layer of a single row of pear-shaped cells, and an outer layer of one or two rows of wedge-shaped cells.

The form of the cells naturally varies with the degree of distension of the bladder.

DEMONSTRATION.

1. Section of Malpighian pyramid transverse to the tubes.

2. Nitrate of silver preparation to show the epithelium of the capsules.

3. Transverse section of ureter (potassium bichromate 2 p.c.; hæmatoxylin). Coats similar to those in the bladder are seen (cp. § 7).

LESSON XXX.

URINE AND ITS CHIEF CONSTITUENTS.

A. NORMAL URINE.

1. Determine the specific gravity of urine by means of the urinometer. It usually lies between 1015 and 1025.

2. Test the reaction of fresh urine with litmus paper; it is usually **acid**, owing to the presence of acid salts (mainly of acid sodium phosphate) and *not* of free acid.

3. Put some urine, say 200 c.c., in a warm place, and observe from time to time.

It will, after twenty-four or more hours, lose its acid reaction, and become alkaline. Gently warm the litmus paper, turned blue by the urine, the blue colour will disappear, showing that the alkalinity is due to the presence of ammonia or a salt of ammonium.

It will gradually become cloudy, and yield a deposit of various salts.

Its odour will become putrefactive.

The urine has undergone *alkaline fermentation*.

4. A small quantity of **mucus** (and nucleo-albumins) derived from the urinary passages is occasionally present in the form of a faint cloudy precipitate. This may be rendered more apparent by the addition of acetic acid.

5. *a.* Add ammonia or caustic potash and warm; a flaky precipitate of **phosphates** of Mg and Ca comes down. Add a few drops of nitric acid, the precipitate dissolves. In neutral urine, earthy phosphates may come down on warming only.

b. Add half its volume of nitric acid, a little ammonium molybdate, and warm; a crystalline yellow precipitate indicating phosphates is formed.

6. Acidulate with nitric acid, and add silver nitrate, a white precipitate, indicating **chloride** falls. Nitric acid is added to prevent the precipitation of phosphates.

7. Acidulate with hydrochloric acid and add barium chloride or baryta mixture (barium hydrate, and nitrate), a white precipitate falls indicating **sulphates**. Hydrochloric acid is added to prevent the precipitation of phosphates.

8. Add to urine about $\frac{1}{3}$ vol. of baryta mixture. A precipitate of sulphates and phosphates falls. Filter this off, test the reaction of the filtrate; it is probably alkaline; neutralise with acetic acid and add a little mercuric nitrate, a white precipitate indicating **urea** is formed (cp. B. below).

9. *a.* Add a little strong hydrochloric acid to about 200 c.c. of urine and let it stand for 12 hrs.,

crystals of **uric acid** coloured brown with pigment form on the sides of the vessel.

b. Acidulate urine in a watch-glass or small shallow vessel with acetic acid, place one or two threads in it and let it remain for twelve hrs., crystals of uric acid form on the threads. Examine the crystals obtained in *a* and *b* (cp. C. below).

10. Examine the original urine with a spectroscope. Observe that no definite absorption bands are to be seen; but only a general absorption of the violet. The chief colouring matter of the urine, *urochrome*, yields no bands.

11. *a.* Add nitric acid; the colour of the urine deepens owing to the action of the acid upon the chromogenic substances.

b. Saturate not less than 200 c.c. of urine with neutral ammonium sulphate. Filter off the precipitate, let it dry, extract it with a little alcohol, a pale brown solution containing some urobilin is obtained. Add a few drops of HCl, the colour deepens on the conversion of chromogen to urobilin. Examine with the spectroscope. Observe a single absorption band situated in the blue green. The middle of the band is about λ 490.

12. Boil urine, no precipitate, coagulable proteids absent (cp. § 5, phosphates), apply general tests for proteids, all proteids absent.

13. Apply Trommer's or Fehling's test to urine. A slight reduction may occur leading to a slight decolorisation of the copper salt, but a distinct red precipitate is not formed. The slight reduction is mainly due to uric acid and creatinin.

B. UREA.

1. **Reactions of urea.** Place a few crystals of urea in a watch-glass, and dissolve them in a small quantity of water.

a. Mount a drop of the solution on a slide, cover with a cover-slip and warm gently, and when it has partially evaporated observe under a high power the *crystals of urea*, consisting of four-sided prisms commonly ending in two surfaces or in a single oblique surface; if the evaporation is rapid the urea crystallises in long spicules.

b. Add to another drop on a slide a drop of pure, strong, nitric acid, cover and warm gently; observe under the microscope the rhombic and six-sided tablets of *nitrate of urea* which crystallise out. Note the striæ frequently present in these tablets.

c. Repeat (b), using a concentrated solution of oxalic acid instead of nitric acid. *Oxalate of urea* will crystallise out in various forms, prominent among which will probably be long, thin, flat crystals, often in bundles; regular rhombic prisms, or tablets resembling somewhat those of nitrate of urea, may also be seen.

d. Dilute considerably the remaining solution, and add to a part of it a solution of mercuric nitrate. A white precipitate of mercuric oxide combined with urea will at once take place.

e. To some of the urea solution add a little sodium chloride and then mercuric nitrate drop by

drop. A certain quantity of the mercuric nitrate must be added before any precipitate is formed; a double decomposition takes place between the mercuric and sodium salts so that as long as any sodium chloride is present, the mercuric nitrate is not available for precipitating the urea.

f. Add nitric acid yellow with nitrous acid, effervescence and evolution of CO_2 and N take place owing to decomposition of the urea.

g. Add sodium hypobromite, effervescence and evolution of gas take place (cp. § 2).

h. Place a little urea in a dry test-tube and heat it carefully over a flame; when the temperature is somewhat over 100°C . it melts and ammonia is given off (recognised by its odour); let the tube cool; add a small quantity of water to dissolve the residue and pour the fluid into another test-tube containing a strong solution of sodium hydrate and a drop of cupric sulphate. A violet colour will be produced, indicating the presence of *biuret*.

2. Quantitative estimation of urea.

a. Knop-Hüfner method. (Hypobromite method.)
Take a burette of 50 c.c. capacity, fit a three-way tube by means of a rubber cork into the top, put a piece of thick rubber tubing carrying a screw clamp on the upper tube, and connect the side tube by small rubber tubing to a small wide-mouthed bottle of about 50 c.c. capacity through a well-fitting rubber cork (cp. Fig. 15). Support the burette *a* on a stand so that it hangs vertically in a cylindrical vessel *b* of about 1.5 litre

capacity containing water. Place the bottle *c* in a shallow vessel of water, supported on the stand at a height which will allow the burette to be raised through a range of about 40 c.c. of its graduation, without the rubber tubing connecting it to the bottle being unnecessarily long. Have ready a small glass bottle or tube *d* of 10–15 c.c. capacity.

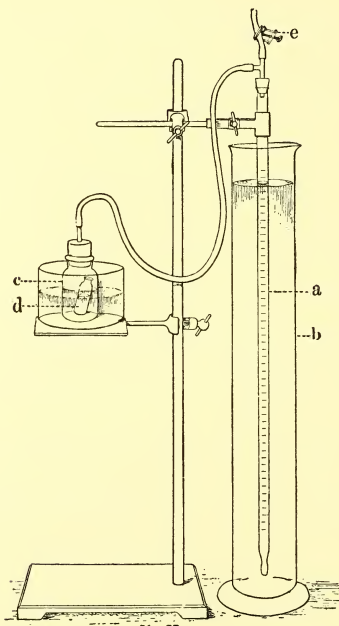
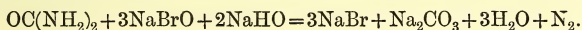


Fig. 15.

Hypobromite solution. Dissolve 100 grams of sodium hydrate in 250 c.c. water, cool, and add gradually 25 c.c.

of bromine, cooling the mixture in a stream of water as the bromine is added. The sodium hydrate solution should be kept ready, and bromine added just before the mixture is required. It is convenient to have thin glass capsules, each containing 2.3 c.c. of bromine; if one of these be placed in 25 c.c. of the sodium hydrate solution, broken by a sharp shake, the proper amount of hypobromite solution for a single observation is obtained.

When sodium hypobromite in an excess of alkali is added to a urea solution nitrogen is given off; thus,



According to this equation 1 gram of urea gives off very nearly 373 c.c. of nitrogen. In fact, however, the decomposition of urea is not complete, the amount of the deficit depends chiefly upon the amount and strength of the hypobromite solution, and on the percentage of urea in the urea solution.

Estimation of the gas evolved. Adjust the level of the water in the vessel *b* (Fig. 15) and in the burette to the zero mark of the burette, the clamp *e* being open. Put 25 c.c. of hypobromite solution into the bottle *c*. Put 5 c.c. of urine accurately measured by a pipette into the small tube *d* and with the aid of forceps carefully put the tube into the bottle *c*. Fit the rubber cork tightly into the bottle. Firmly close the clamp *e*. Tilt and gently shake the bottle, so that the urine and hypobromite solution mix. As the gas comes off the water is depressed in the burette. When the evolution is ended raise the burette until the water level within and without the burette is the same.

Leave for five minutes, then readjust the level of the water and read the volume of nitrogen given off.

Calculation of percentage of urea. Theoretically 373 c.c. of nitrogen are given off by 1 gram of urea, if then v c.c. of nitrogen, after correction for temperature and pressure, are obtained in an experiment, this will have been given off by $\frac{v}{373}$ grams of urea, that is there are $\frac{v}{373}$ grams of urea in the 5 c.c. of urea solution taken, so that the percentage of urea in the solution is $\frac{20v}{373}$.

But since in practice the whole of the nitrogen is not given off, the deficit must be allowed for; if we take Hüfner's estimate of the deficit of the volume of the nitrogen, viz. 4.4 p.c., the percentage will be $\frac{20v}{357}$.

Correction for temperature and pressure. Since the theoretical amount of nitrogen is estimated at a temperature of 0° C. and a pressure of 760 mm. of mercury, the volume of the gas obtained must be corrected for this temperature and pressure as follows. If v' be the volume in cubic centimetres of the nitrogen obtained, at temperature t° C. and pressure B in mm. of mercury, and T be the tension of aqueous vapour at t° C., the volume v at 0° C. and 760 mm. pressure will be

$$\frac{v'}{1 + .003665 t} \times \frac{B - T}{760},$$

.003665 being the coefficient of expansion of gases.

Sources of error in estimating urea in urine. Nitrogenous bodies other than urea are partially decomposed. Further

certain substances increase and others decrease the completeness of the decomposition. Hüfner, Pflüger and Schenk find with the apparatus used by them, and with a 1 p.c. solution of urea, that the deficit of nitrogen is very nearly 4.4 p.c., *i.e.* 1 gram of urea instead of giving off 373 vols. of nitrogen gives off 357.

Russell and West using a 2 p.c. solution of urea find with their apparatus a deficit of nearly 8 p.c., *i.e.* 1 gram of urea, instead of giving off 373 vols. of nitrogen gives off about 343 vols. When the volume of the gas is measured at a temperature of 18° C., no correction being made for the tension of the aqueous vapour, the deficit of 8 p.c. is nearly corrected by the expansion of the gas, so that according to Russell and West, in making an approximate estimate of urea with their apparatus, no account need be taken of a deficit of nitrogen, nor any correction be made for temperature and pressure.

When, as in medical practice, a rough estimation of the percentage of urea is sufficient, the tube in which the nitrogen is collected is graduated empirically, at the ordinary indoor temperature, *i.e.* at about 18° C. The graduation is performed by mixing a known quantity of hypobromite solution with 5 c.c. of a 1 p.c. solution of pure urea, and marking on the collecting tube the volume of nitrogen given off. Similar observations are made with 5 c.c. of a 2 p.c. and of a 3 p.c. solution of urea. The tube, between each of the marks so obtained, is divided into tenths. Hence, when 5 c.c. of a solution of urea of unknown strength are taken, and treated as the standard solution was treated, the percentage of urea in it is at once roughly determined by the number of divisions of the tube occupied by the nitrogen given off.

b. Liebig's method. Take 40 c.c. of urine and add 20 c.c. of the baryta mixture; a copious precipitate of barium phosphate, sulphate, carbonate will be formed. Shake to mix well the fluids

and filter through a dry filter. The object of this is to remove the phosphates, these give a precipitate with mercuric nitrate.

Fill a burette with the standard solution of mercuric nitrate. (Cp. p. 375.) On a glass plate lying on a dull black surface place a number of drops of a saturated solution of normal sodium carbonate.

Preliminary estimation. In a beaker place exactly 15 c.c. of the filtered urine-mixture, into this run slowly the mercuric nitrate solution from the burette. Stir with a glass rod, and from time to time add a drop of the mixture to one of the drops of sodium carbonate; as soon as a yellow colour is thus produced note the amount of mercuric nitrate which has been added. The yellow colour is produced when all the urea has been precipitated and there is an excess of mercuric nitrate; this with sodium carbonate gives a yellow precipitate of oxide of mercury or of a basic mercuric carbonate.

Second estimation. In the preliminary estimation too much mercuric nitrate will probably have been added; so the process should be repeated, adding at once the amount of mercuric solution found in the preliminary estimation less 1 c.c. After well mixing, a drop is added to a drop of sodium carbonate; if a yellow colour results, the process must be repeated, adding less mercuric nitrate; probably however the mixed drops will be colourless, if so add .1 c.c. mercuric nitrate, mix and test again, and so on adding .1 c.c. at a time until a yellow colour is obtained.

Correction. Since the sodium chloride in the urine prevents the precipitation of urea by mercuric nitrate (cp. § 1, e) more mercuric nitrate is added to the urine than is required to precipitate the urea in it. The excess thus added is found experimentally to be about 2 c.c. for 10 c.c. of urine. *Hence 2 c.c. must be deducted from the number of c.c. of the standard solution actually used.*

If v be the corrected number of c.c. of mercuric nitrate used, the 10 c.c. of urine taken contain $\cdot 01 v$ grams of urea, *i.e.* the percentage of urea in the urine is $\cdot 1v$.

Note. The method as above given is very rough. For an

accurate estimation, the sodium chloride as well as the phosphates must be separated from the urine, the filtered mixture must be neutralized, and again neutralized with sodium carbonate after each addition of mercuric nitrate. It must also be remembered that the amount of urea found by this method is too high, since ammoniacal salts and all the nitrogenous bodies in the urine give a precipitate with mercuric nitrate.

C. URIC ACID.

1. Reactions of uric acid.

a. Place a few crystals of uric acid on a slide, and add a little strong sodium hydrate to dissolve them; then add nitric acid just to excess. Examine with a high power the crystals of uric acid formed. They are generally rhombic plates with obtuse angles, but they vary greatly in form. Note the star-shaped and dumb-bell crystals.

b. Make a saturated solution of uric acid in strong sodium hydrate, mount a drop and place it aside for some time. Crystals of sodium urate will crystallise out. Note the spheres covered with small thin projecting prisms; hexagonal prisms and thick tablets may also be seen.

c. To a little uric acid in an evaporating dish add a little fairly strong nitric acid, the uric acid will be split up and gases given off. Evaporate to dryness on a water bath at 100°C . but finishing at a temperature not above 40°C . A reddish residue will be left, to this add a drop or two of *weak* ammonia; a red-purple colour will be formed; this is the **murexide test** for uric acid. Add then a drop of strong sodium or

potassium hydrate, the colour will turn to a blue-purple.

d. To a dilute solution of uric acid in strong sodium hydrate add a drop of cupric sulphate (or Fehling's fluid) and boil; a reduction of the cupric sulphate will take place (cp. p. 247, § 13).

e. Test the solubilities of uric acid by treating some with the fluid, filtering and evaporating the filtrate to dryness; it will be found to be insoluble in water (except in traces), insoluble in alcohol, slightly soluble in boiling water. Add strong sulphuric acid to a little uric acid, it will dissolve readily.

2. *Quantitative estimation of uric acid.* Take 100 c.c. of urine accurately measured, add to it crystals of ammonium chloride to saturation, about 30 grams will be required. Let the beaker stand for two hours, filter off the precipitate of ammonium urate and wash it with a saturated solution of ammonium chloride. Remove the precipitate from the filter with a jet of hot water, add five drops of strong hydrochloric acid, heat just to boiling; free uric acid is formed.

Let it stand 12 hrs. Filter through a small filter. Measure the filtrate. Wash the uric acid with cold distilled water until the washings are free from chlorides. Then wash the uric acid off the filter into a weighted crucible, evaporated to dryness on water-bath at 100° C., dry in air-bath at 110° C., cool and weigh. To the weight of uric acid found add 1 mgr. for each 15 c.c. of the measured filtrate, since uric acid is soluble to this degree in water.

D. ABNORMAL URINE.

1. Determine its specific gravity (cp. with sp. g. of A. § 1).

2. Test its reaction. If alkaline, determine by smell whether ammonia is present or not.

3. If the urine is alkaline or neutral just acidulate it with acetic or nitric acid and boil. A precipitate indicates the presence of **coagulated proteids** (albumin, or globulin, or both). Add a few drops of nitric acid; the coagulated albumin does not dissolve. If the urine is neutral or only slightly acid earthy phosphates may be precipitated on heating, but the precipitate dissolves readily on the addition of nitric acid (cp. A. § 5). If much albumin or globulin is present, the addition of strong nitric acid without heating causes a precipitate of coagulated proteid.

4. Saturate the urine with ammonium sulphate, thus precipitating coagulable proteids together with pigmented urates. Heat on the water bath for $\frac{1}{4}$ hour to coagulate completely albumin and globulin. Then apply the biuret test to the filtrate for *peptone*, and to the aqueous extract of the precipitate on the filter for *albumose*.

5. *a.* Coagulate any albumin present, taking care to only just acidulate, and filter, apply Trommer's or Fehling's test. A definite red or yellow precipitate forming immediately, or after standing for 5 minutes, indicates the presence of **sugar** (dextrose) in abnormal quantity.

b. Quantitative estimation of dextrose. Since diabetic urine is usually rich in sugar, take 20 c.c. of the urine and dilute it to 100 c.c. with water, then make a preliminary estimation of the sugar with Fehling's fluid (cp. p. 181, § 9). If more than 1 p.c. is found in the diluted urine, dilute it still more, so that the amount of sugar lies between .5 p.c. and 1 p.c. and make a second estimation. Calculate the percentage present in the original urine.

6. If the urine is of a deep colour, dark brown or greenish, test for **bile pigments**.

a. By Gmelin's test (cp. p. 204, § 3). The green stage only of the test is conclusive; substances other than bile pigments may give the red and bluish tints.

b. Saturate the urine with ammonium sulphate, filter, extract the precipitate with hot alcohol, which dissolves the pigments, filter; to the alcoholic extract add strong sulphuric acid and warm gently. A rich blue-green colour indicates bile pigments.

7. Test for **bile-salts** as follows :

Dilute the urine 10 times with water, add one drop of .1 p.c. furfural solution for each c.c. of fluid ; pour strong sulphuric acid down the test-tube. A purple ring at the junction of the liquids (it may form only on standing) indicates bile-salts.

8. The presence of **blood** in urine in small amount gives it a characteristic reddish-brown colour. Examine it spectroscopically, if blood is present the bands of oxyhæmoglobin will be seen. Methæmoglobin and other derivatives of hæmoglobin are sometimes present in addition. If much blood is present the urine is deep red in colour and red corpuscles may be detected microscopically.

If the bands are too faint to be identified with certainty, add a little NaHO and boil. Cool the fluid and add a few drops of ammonium sulphide ; the bands of hæmochromogen (cp. p. 237) will be seen if hæmoglobin was present.

9. The presence of **pus** in urine leads to more or less white sediment. Examine the sediment microscopically, characteristic colourless cells are seen, the nuclei of which become obvious on irrigation with 1 p.c. acetic acid (cp. pp. 4, 10).

10. In addition to red corpuscles and pus cells, urinary sediment may show microscopically the presence of **formed elements**, such as epithelial cells, spermatozoa, entozoa, micro-organisms.

11. The commoner **crystalline deposits** in urinary sediment are identified as follows:

a. urates. These are often deposited in normal urine on cooling; they are pinkish in colour and dissolve on warming. Star-shaped clusters of acicular crystals occur but the sediment is largely amorphous.

b. uric acid. This appears as small dark reddish crystals on the sides of the vessel. Observe the crystalline form (cp. C. 1, *a*). Apply the murexide test to the crystals.

c. phosphates are present only in alkaline urine; the precipitate increases on warming and dissolves on the addition of acid. The crystalline forms are prismatic and feathery stars (ammonio-magnesium phosphate), and prismatic plates in rosettes, spherules and dumb-bell shaped (calcium hydrogen phosphate).

d. calcium oxalate, occurring as octohedra, and having an envelope-like appearance.

LESSON XXXI.

THE DUCTLESS GLANDS.

1. Section of **thyroid** of cat or other mammal. (Flemming's fluid, stained in bulk, imbedded.) Note

The separate roundish **vesicles** forming the gland ; they vary much in size, and have very little connective tissue between them.

The epithelium of the vesicles, the cells are of varying height, but usually somewhat flattened.

The homogeneous **colloid substance** filling the central space of the vesicles (the colloid is stained yellow with picric acid).

2. Section of *parathyroid* of cat (treatment as thyroid in § 1). Note the cells arranged in curved cylinders, or columns, or roundish masses ; there are no vesicles like those of the thyroid and no colloid substance.

3. *Pituitary body of cat.* (Flemming's fluid.) Stain with Ehrlich-Biondi fluid, or with borax-methylene blue and eosin. Transverse sections of anterior and of posterior lobe cut separately, frozen (the posterior lobe is apt to break into pieces).

Note in the *anterior lobe* the anastomosing cell-tubes, or columns of cells ; the two forms of cells, viz. ovoid, deeply staining cells, generally peripherally placed, and smaller cubical or columnar cells ; here and there, especially at the outer portion, stained secretion may be seen in the lumina of the tubes.

Note in the **posterior lobe** a peripheral cap of clumps of cells which may anastomose; here and there a clump surrounds a homogeneous mass.

4. Section of **supra-renal body** of cat¹, cut transversely to its long axis. (Flemming's fluid, cut frozen.) Stain with hæmatoxylin (better with Ehrlich-Biondi mixture). Note

The connective tissue capsule.

The **cortex**, consisting of bands of cells stretching inwards from the capsule. The **medulla** fairly sharply marked off from the cortex; it consists of a network of rather tubular-looking cell-masses, and generally shows some conspicuous, thin-walled veins.

If stained with Ehrlich-Biondi mixture, the cortex will be a darker red than the medulla; if the gland is hardened in mercuric chloride and stained with picocarmine the cortex will be yellowish, and the medulla red.

5. Distinguish the zones of the cortex, viz. (1) an outer narrow zone, the zona glomerulosa, consisting of *cylinders of cells*; at right angles to the surface there are usually two elongated cells from side to side of the cylinder, and the nuclei are nearer the centre than the basement membrane; (2) a broad zone, zona fasciculata, consisting of *cell-columns* continuing the line of the cylinders but for the most part having a single row of polyhedral cells. (The arrangement resembles somewhat that of the liver cells.) The columns here and there communicate; (3) a narrow zone, the zona reticulata, consisting of a *cell-plexus*, generally two cells from side to side of a strand; the cells are polyhedral and smaller than those of the preceding zone.

The cells of cortex, especially those of the outer part of the cell-columns, contain usually a number of small fat globules; in the section they may have been dissolved by the alcohol; if so

¹ The appearance varies somewhat in different mammals.

the cells under a high power will appear to have a very distinct network.

6. Tease out a piece of fresh supra-renal (*a*) from the cortex, (*b*) from the medulla. Note that the cells of the cortex are granular or contain fat globules, those of the medulla are transparent; irrigate with potassium bichromate 1 to 2 p.c., the cortex cells become yellow, the medulla cells become reddish-brown.

7. Section of a lobule of the **thymus** of a new-born animal. (Mercuric chloride; picrocarmine.)

a. Note under a low power

The connective tissue sending in trabeculæ and dividing the **cortex** into alveoli. The alveoli are crowded with leucocytes.

The central mass or **medulla** of the lobule staining less than the cortex. In it are some small bodies concentrically striated, the **corpuscles of Hassall**.

b. Note under a high power

In the alveoli of the cortex there is no indication of a lymph channel (cp. p. 213).

In the medulla, small leucocytes are relatively less frequent than in the cortex; connective tissue cells with membranous processes and giant-cells may be seen; the striation of the corpuscles of Hassall is due to layers of flattened cells.

DEMONSTRATION.

Section of thymus with blood vessels injected. Note that in the cortex the capillaries run, broadly speaking, in loops between the periphery and the centre of the alveoli. (Cp. with lymphatic gland, Lesson xxv.)

LESSON XXXII.

SKIN. TOUCH.

A. SKIN.

1. Vertical section of human scalp¹. Stain with picrocarmine. Observe

a. The **epidermis**; proceeding from the outside inwards.

The **horny layer**, consisting of much flattened cells, without visible nuclei, possibly the inner part only of this will remain.

The **stratum lucidum**, a thin layer of transparent cells (stained yellow by picric acid).

The **granular layer**, one or two cells deep, probably stained deeply with carmine. Examine under a high power for the eleidin granules of the cells (with some methods of hardening they may not be seen).

The **Malpighian layer**, consisting of (*a*) polyhedral cells (prickle cells), and of (*b*) a row of columnar or elongated cells, the germinal layer.

¹ Either mercuric chloride, alcohol, picric acid, and formol, or chromic acid may be used for hardening skin.

b. The **dermis**, consisting of white, fibrous, and elastic tissue, which becomes coarser and has larger spaces in passing inwards. Note the **papillæ**, conical projections of the dermis surrounded by epidermis. A thin, fairly homogeneous basement membrane may be seen beneath the epidermis; it is not always distinct.

c. The **sub-cutaneous connective tissue** continuous with and indistinguishable from the deep layer of the dermis. It consists of loose connective tissue, with, usually, masses of fat-cells.

d. The **hairs**. Before examining these in the section, take a hair from the head, cut off a small piece, place it in 95 p.c. alcohol containing picric acid, leave a few minutes, wash in strong alcohol, clear and mount. Note in this the outer hyaline cuticle of the hair (here and there yellow stained scales will probably be seen partly detached from it), and the core with longitudinally arranged pigment grains; possibly also with a central portion, the pith. Turn now to the section, and follow a hair cut longitudinally in its lower part, it expands at the end forming the **hair-bulb**. Note

The dermis sends the **papilla**, a bulbous mass with a narrow neck, into the hair bulb. In the epidermis the hair is surrounded by a continuation of the horny layer; this ceases to be distinct a short way from the surface (a little above the duct of the sebaceous gland). The continuation of the Malpighian layer of the skin outside the hair, the **root sheath** stretching down to the hair bulb.

The **dermic coat** of the hair follicle (the depression in the skin in which the hair lies), formed externally of

dense connective tissue, internally of a hyaline membrane (the hyaline membrane varies in distinctness).

Note under a high power that the hair bulb consists of an outer row of columnar or cubical cells and of inner polyhedral cells, and that the cells are continuous laterally with the root sheath and centrally with the hair.

e. The **sebaceous gland** (or glands) attached to each hair. It consists of a duct, and of a variable number of alveoli. The duct opens into the upper part of the hair, its epithelium is continuous with that of the root sheath. The alveoli consist of roundish masses of polyhedral cells in which the cell substance appears as a network owing to the solution of the fatty globules it contained.

f. The **erector muscle of the hair**. The erector stretches as a band of unstriated muscle from the upper part of the dermis obliquely inwards to the dermic sheath of the hair, a little below the sebaceous gland.

g. One or two sweat glands may be present, but they will be better seen in § 2.

2. Section of the tip of the finger; stain with hæmatoxylin. Note in the **sweat glands** the gland duct, a small tube showing in the dermis numerous nuclei; it consists of two or three layers of cells continuous with those of the Malpighian layer of the epidermis. In the epidermis the lumen of the duct is continuous with a tubular and more or less spiral channel between the cells.

The terminal secreting tube in the sub-cutaneous

tissue. This forms a coil, and hence will be seen as a clump of tubes cut in various directions. The secreting portion is larger in diameter than the duct, it is lined by a single layer of cubical or columnar cells (a layer of longitudinal fibres may be present between the cells and the basement membrane).

3. Sensory nerve-endings in stratified epithelium. Vertical section of cornea of cat or rabbit. (Gold chloride .5 for an hour; cp. Lesson VII. § 6; alcohol; cut in paraffin.) Observe

The bars of the primary plexus (cp. Lesson XIV. § 11) cut across; from this plexus small bundles of fibrils run through the anterior membrane and separate into a brush of fine varicose fibrils which spread out immediately underneath the epithelial cells forming the **sub-epithelial plexus**. Probably the section in some part will be a trifle oblique; here a fragment of the small meshed sub-epithelial plexus will be seen, elsewhere fine fibrils will be seen running a longer or shorter course underneath the cells¹.

Between the epithelial cells of the cornea the **epithelial plexus** of very fine varicose nerve fibrils; in places the fibrils will be seen to run from the sub-epithelial plexus.

The peripheral course of the nerve fibres in the parts of the skin where there are no special terminal organs is in the main like that described above for the cornea.

¹ The sub-epithelial plexus is only seen satisfactorily in sections cut from the surface of the cornea which include the anterior surface of the basement membrane.

4. Examine again the skin of the tip of a finger (§ 2). Observe the **touch corpuscles**.

They are oval in form, and are found in the axes of the papillæ; many papillæ are without them, in which case a capillary may generally be seen running up into the papilla.

They appear to consist of a mass of connective tissue in which nuclei are disposed transversely. (For the nerve fibre cp. Demonstrations, § 2.)

Pacinian bodies (cp. § 5) may sometimes be seen in the sub-cutaneous tissue.

5. Examine the mesentery in a recently killed cat; in it will be seen a considerable number of oval transparent bodies, the **Pacinian bodies**. (Some are always present in the mesentery just above the inferior mesenteric artery.) Select one which is not surrounded by fat, cut it out and mount it in normal saline solution. With a little care the mesentery covering it may be removed with needles. Observe

The medullated nerve fibre and the thickening of its connective-tissue sheath as it approaches the Pacinian body.

The division of the connective-tissue sheath to form the numerous concentric capsules, which become closer together towards the centre of the body. At intervals on the capsules elongated nuclei will be seen.

The loss of medulla as the sheath splits up.

The hyaline core inside the innermost capsule.

The axis cylinder penetrating the core, and ending in it in a slight enlargement.

6. Treat a piece of skin of the face of a rat or kitten (containing the whiskers) with gold chloride and formic acid

(Lesson XVI. § 5). Harden in alcohol, imbed, cut sections. Note the nerve plexus and the sub-cutaneous tissue and the bundles of nerve fibres which proceed from it to the large hairs (*sinus* or *tactile hairs*).

Note further the superficial nerve plexus in the upper part of the dermis, and the small bundle of nerve fibres sent downwards to the smaller hairs ; the nerve fibres form a ring around the hair just below the duct of the sebaceous gland.

7. Transverse section of roots of sinus hairs of rat or young kitten (osmic acid, cut in paraffin). Note the transversely cut bundles of medullated fibres arranged in a circle in the root sheath.

8. Section¹ of the beak of a young duckling (Flemming's fluid, picrocarmine). Note

The simple form of touch corpuscles (Grandry's corpuscles) just below the epidermis, consisting of two or three disc-shaped cells to which a medullated fibre runs, ending in a non-medullated expansion between each two cells.

The simple forms of Pacinian corpuscles (Herbst corpuscles) ; they are smaller than Pacinian bodies and have fewer capsules ; a row of nuclei is seen in the core on either side of the axis cylinder.

B. TOUCH.

NOTE. In many experiments on touch, taste, and smell, two students should work together. One (*A*) should close his eyes, or be otherwise prevented from seeing what is going on, the other (*B*) should apply the tests to him, and record the results. At the end or midway in the experiment they change rôles.

1. Threshold for two points (tactile spatial discrimination). It will be sufficient for the student to

¹ Perhaps best seen in a transverse section of the posterior part of the bill.

determine the threshold roughly, within a centimetre in the case of the fore-arm. *A* sits with the fore-arm bare, and extended on the table, palm uppermost; eyes being shut.

B has a pair of compasses and a millimetre scale; he separates the points 3.5 cm. and touches *B*'s arm either with one point or with both points. The two points must be applied with equal pressure, and both at the same moment, and they should be applied lengthways to the arm. *A* says whether he feels one point, or two points, or is doubtful whether it is one or two; *B* writes down after each observation whether he applied one point or two points, if two their distance apart, and *A*'s statement.

If *A* is right three times out of four with the single point and the two points, *B* without telling *A* puts the points of the compasses at 3.0 cm., and makes similar observations; then with the points 2.5 cm. apart, and so on. Probably *A* will be unable to distinguish two points when they are 2.0 or 2.5 cm. apart. The minimal distance at which they can be distinguished, say four out of five times, is the threshold.

If the points are separated a little less than the threshold, they will probably be felt as two if they are more down the arm, or if they are put transversely to the arm.

When the approximately minimal distance at which *A* does not distinguish the two points, he is told to open his eyes, and observe the application of the points.

A shuts his eyes once more, and the points are applied to the tip of the forefinger; the points being at first 1 cm. apart, then .75, and so on; now and

then a single point being applied. The threshold will probably be less than .5 cm. A opens his eyes, the points are applied at a distance a little above the threshold.

Similar observations may be made on other parts of the body.

If the threshold is determined for the back of the neck, the two points being applied on either side of the mid-line, it will probably be found that they are not distinguished if they are applied longitudinally, or both on one side of the neck.

2. Cold spots in the skin. Take two metal rods 1 to 1.5 mm. in diameter and rounded at the ends and dip them into a freezing mixture or into ice and water, dry before using (the rod may also be cooled) by dipping it into ether, and allowing the ether to evaporate. Mark out with dilute Bismarck brown an area about 2 cm. square on the fore-arm. Lightly press the end of a rod on one corner of the enclosure for 2 secs.; if it is felt as cold mark it brown, if not place the rod on the immediately adjoining spot of skin, and so along the side of the square, marking all the points felt as cold. Map out similarly line after line of the square. It will be found that in some places cold is felt keenly and at once, in other places dully, and in others not at all.

Test the cold spots again, and mark the points most sensitive to cold with methylene blue. In re-testing, an interval of a few seconds must be allowed between two applications to the same spot.

3. Sensation of heat in different parts of the skin. Dip a small metallic knob (or a bulb of a

mercury thermometer) into water at 65°C. to 70°C. , quickly dry it, and apply it to the fore-arm, and note the sensation. Warm it again, and apply it to the forehead, the sensation of heat will probably be much greater than in the preceding case. Compare similarly, the tip of the finger, the cheek, and the palm of the hand and the back of the neck.

4. *Warm spots in the skin.* Proceed as in § 1, but warm the rods in water to 70°C. , or as hot as can be borne without pain, and mark out the spots where heat is felt. The results will probably be much less precise than in the case of the cold spots.

5. **Successive temperature contrast.** Put one hand into water of 40°C. and the other into ice-cold water. After a minute put both into water of 20°C. This water will feel warm to the hand which has been in the cold water and cold to the hand which has been in the hot water.

Or,

Put one hand into water of 20°C. It will feel cold. Now keep the hand in water of 10°C. for one minute and again put it into the water of 20°C. It now feels warm.

6. **Effect of temperature as estimation of weight.** Place two metal discs of equal size and weight, one cold, the other warm, on corresponding fingers of the hand; the cold one will feel the heavier. Place both on the forehead, and estimate the relative weights.

7. **Estimation of weight with and without the aid of the sense of movement (muscular sense).** A sits with hand out-

stretched at a table and with eyes shut. *B* has metal discs of equal size of 10, 11, 12, 13, 14, 15 grams. He takes 10 grams and 13 grams, and placing first one and then the other in the palm of *A*'s hand, writes down which, if either, *A* feels to be the heavier. If *A* is wrong 3 times out of 4, then 10 and 15 grams are compared; if *A* is right 3 times out of 4, the 10 and 12 grams are taken, and so on. Having determined approximately the difference which can be distinguished in light weights, heavier weights are taken, viz., 300, 330, 360, 390, 420, 450 grams, and the power of distinguishing these is tested in the same way. These weights should be tin cans with handles, of equal size, 6 to 8 cm. in diameter, the weight being made up with shot.

If with the lighter weights 10 and 12 grams could be distinguished, but not 10 and 11, then with the heavier weights, probably 300 and 360 will be distinguished, but not 300 and 330.

B now gives to *A* the 300 gram tin, and the tin just below the threshold for the pressure sense; *A* takes them one in each hand, and lifts them up and down, and changes them from one hand to the other. He will probably then be able to detect a difference he was previously unable to detect.

These experiments require some practice; it may be found that a sense of difference of weight is distinct at one moment, and disappears the next.

8. Localization. (*a*) *A* places one hand, say the left, on the table with digits outstretched, the right arm being extended from the side and the eyes shut. At the call of *B*, *A* attempts to touch with the forefinger of the right hand the tip of the finger called. *B* marks on a sketch the result.

(*b*) *B* marks 5 spots at some distance from another on *A*'s left fore-arm, and makes a sketch of the arm and the spots. *B* touches one of the spots, and *A* immediately tries to put his right forefinger on it. *B* marks in the sketch the results.

9. **Stimulation of nerve trunk and of nerve-endings.** Place the elbow first in warm water, and then in a mixture of ice and water. There will be a sensation of pain in the fingers and cold in the elbow. The application of cold to the trunk of a nerve does not cause a sensation of cold.

10. **Tactile illusions.** Cross the second finger over the first or the third over the second, and place between their tips a small marble (or any solid round body a little larger than a pea) so that it touches the radial side of the first, and the ulnar side of the second finger-tip. On gently rolling the body about, a sensation as if of two distinct bodies will be felt.

The same illusion is experienced if the tip of the nose be gently rubbed with the tips of the fingers so placed.

DEMONSTRATIONS.

1. Vertical section of scalp, or other skin containing hairs (osmic acid). Note the fatty globules in the sebaceous glands.

2. Vertical section of tip of finger (osmic acid). Note the medullated fibre entering a touch corpuscle.

3. Section of skin of face of rat or cat cut parallel to the surface (alcohol, picrocarmine). Observe one of the large tactile hairs with its cuticle, and the sheaths of its follicle (cp. A. § 1, *d*).

4. Section of beak of duckling. Note the simple form of touch corpuscles (cp. A. § 8).

5. Piece of mesentery of cat containing Pacinian bodies treated with osmic acid and mounted in water (or dilute glycerine). Note the bundle of nerve fibres separating to the several Pacinian bodies. Some of the fibres may divide and supply more than one Pacinian body.

6. Pacinian body after removal of the mesentery treated with silver nitrate. Note the outlines of the epithelioid cells of the outer capsules.

7. Longitudinal and transverse section of bed of nail (picric acid; picrocarmine). Note the upper clear portion of the nail (corresponding to the *statum lucidum* of the skin), the Malpighian layer, and the longitudinal ridges of the dermis.

8. Skin of mammal with blood vessels injected.

9. Pencil for detecting heat and cold points.

10. Instrument for testing pain (Algometer). In this instrument a blunt point is pressed with variable and known pressure against the skin.

LESSON XXXIII.

TASTE AND SMELL.

A. TASTE ORGANS. THE TONGUE.

1. Longitudinal section of a papilla foliata of the tongue of a rabbit (chromic acid .2 p.c. or potassium bichromate 2 p.c.). Stain with hæmatoxylin and eosin. Observe

The transverse section of the ridges with the intervening fossæ.

In each ridge three papilliform processes of the dermis, one median, two lateral.

The horny epidermis, consisting of cells very flattened but still showing nuclei, covering the ridges and lining the fossæ.

The Malpighian layer of the epidermis. In the fossæ both layers of the epidermis are thinner and less marked off from one another than on the surface.

On the outside of each lateral papilla, and therefore lining each side of the fossa, the flask-shaped **taste-buds**. There are usually four nearly in contact, one above the other. Each bud is in contact by its deeper part with the dermis of the papilla, and has a

short neck running to the free surface of the epidermis of the fossa, where its circular open mouth may often be seen. The epidermic cells are flattened around its median portion, forming a nest for it.

The external or **cover-cells** of the taste-bud, curved and flattened, with conspicuous oval nuclei.

The internal **taste-cells**, each with spherical or ovoid nucleus, thin peripheral and still thinner central process.

Nerve fibres running along the dermis of the papillæ, and branching off to the buds.

2. Longitudinal section of tip of **tongue** of kitten (Flemming's fluid). Stain with picrocarmine. Observe

The filiform papillæ of the dorsal surface. If fungiform papillæ are also present, note the taste-buds in them.

The absence of papillæ from the ventral surface of the tongue.

The bundles of striated muscular fibres, vertical, horizontal and transverse.

The small glands.

B. NASAL MUCOUS MEMBRANE.

1. Transverse section of nose of newt¹. Observe (l. p.)

The nasal chamber on either side surrounded by

¹ The upper jaw of a pithed newt is cut off in front of the eyes, the anterior part is cut away, the cut passing a little behind the nostrils. The nasal portion is hardened in Flemming's fluid, decalcified, stained in bulk with picrocarmine or with alum carmine, cut in paraffin.

bony and cartilaginous plates and lined by an epithelial layer, thick, except in its lateral part.

The glands and the cut bundles of nerve fibres below the mucous membrane.

Observe (h. p.)

The outer layer of the olfactory epithelium, without nuclei, and appearing as very closely packed thin cell bodies or processes (the outer portions of the columnar and of the olfactory cells).

The inner layer, in which little is seen but closely packed nuclei; the outermost layer of nuclei (belonging to the columnar cells) are elongated; the rest are round or nearly so (nuclei of the olfactory cells).

The bundles of non-medullated fibres of the olfactory nerve, and the small albuminous glands in the sub-mucous tissue. Here and there a duct may be seen running through the epithelium, as a fine tube consisting of basement membrane and flattened cells.

The respiratory epithelium, consisting of columnar ciliated cells and goblet cells. (This will probably be seen in the lateral part of the nasal cavity. It is the sole epithelium at the nasal openings.)

2. Take a pithed frog or newt, cut off the upper part of the head just in front of the eyes, from this cut off the tip a little behind the nostrils. Examine the piece with a lens and observe the nasal cavities, cut away the roof of one nasal cavity, place in osmic acid .5 p.c. for about $\frac{1}{2}$ an hour, wash well with water, place in picrocarmine for 1 to 24 hours (better the longer time), wash, scrape off a little of the olfactory mucous membrane, tease and mount in dilute glycerine. Observe

The **columnar cell**; considered to be a supporting cell; it has a cylindrical outer portion, and a much thinner and sometimes branched inner portion.

The **olfactory cell**; it has a projecting spherical nucleus, a slender, smooth, outer portion, and a still more slender, rather irregular inner portion.

The columnar cells surrounded and partially hidden by three, four, or more olfactory cells.

Probably the granules of the albuminous glands beneath the epithelium will be preserved and be very obvious.

C. TASTE.

See Note under Touch, p. 268.

1. **Rough determination of threshold for the tip of the tongue.** Take the following aqueous solutions, label each set 1, 2, 3, 4, 5; No. 1 being the strongest solution.

Cane sugar 6, 3, 1·5, ·75, and ·375 p.c.

Sodium chloride as cane sugar.

Fuming sulphuric acid, 1, ·5, ·25, ·05, ·01 p.c.

Sulphate of quinine, ·2, ·1, ·02, ·004, ·0008 p.c.

Have ready, small pipettes of equal size, one for each set of solutions (the pipette must be washed out with water if it is used for a weak solution after a strong one); a dish of water to clean out the pipettes; an additional pipette with pure water; a tumbler of water to rinse out the mouth.

B lets fall a drop of one of the weaker solutions (No. 4) or of water on the tip of *A*'s tongue; after 5 seconds *A* says what he thinks it is, and this is noted

by *B*. *A* takes a mouthful of water and ejects it, preventing the test substance so far as possible from coming into contact with the back of the tongue and soft palate.

This procedure is repeated till all the no. 4 solutions and water have been tested.

Then No. 3 solutions and water are similarly tasted; and so on. When it is clear that a solution is distinctly tasted, no stronger solution of that substance should be applied. It will probably be found that a strong solution of cane sugar must be applied before it is tasted; a rather less strong solution of salt; a still less strong solution of acid; whilst a relatively weak solution of quinine will be effective.

2. Comparison of sensitiveness to bitter substances of the tip and of the back of the tongue.

Take the solution next below that which gives a bitter taste on the tongue. Put two drops on the tip of the tongue and rub the tip against the lip; as soon as it is clear that it is not tasted put two drops on the back of the tongue and press the back of the tongue against the soft palate. It will usually be tasted at once.

3. Determine roughly in the manner given in § 1, the threshold for the various substances when placed in the back of the tongue. In these experiments care should be taken, so far as possible, to rinse out the mouth without pressing the back of the tongue against the roof.

4. Take a few c.c. of the solution of sugar next below that which is tasted on the left of the tongue, and roll it about in the mouth, it will taste sweet. This is largely due to a greater area being stimulated,

and perhaps also to some part of the mucous membrane being more sensitive to sweet than the tip of the tongue.

5. Take any of the No. 1 solutions, and place a drop upon the middle of the upper surface of the tongue, it will not be tasted, provided it does not spread to other regions.

6. Arrange for a constant current with two Daniell's cells. Apply one electrode to the middle region of the upper part of the tongue, the other electrode to the back of the tongue. When the anode is on the back of the tongue an acid taste with some bitter will be set up there; when the kathode is applied to the back of the tongue the taste will be alkaline with some bitter. On applying the electrodes elsewhere, probably the acid and alkaline sensation will alone be felt.

7. Lightly press the point of a needle on the tongue, and note approximately the degree of pressure at which touch passes into pain. Then apply to the tip with a camel-hair brush a 1 p.c. solution of cocaine, repeating if necessary. At a certain stage touch will still be felt, but not pain.

8. Apply an extract of gymnema with a camel-hair brush to the back of the tongue (10 grams of the leaves of gymnema silvestris pounded, boiled for 5 minutes in water and filtered); leave for about 30 secs.; wash out the mouth; test the taste sensations with the stronger solutions; the acid and salt will still be tasted, but not quinine or sugar. The solutions must of course not overpass the regions to which gymnema has been applied.

D. SMELL.

Threshold for Smell. Prepare standard solutions of camphor thus: dissolve .1 gram. of camphor in strong spirit, dilute with water to 200 c.c. Put a little of this solution into a test-tube and label (1). Take 1 c.c. of (1) and add 4 c.c. of water, label this (2). Take 1 c.c. of this, add 4 c.c. of water and label (3), and so on till 9 solutions are obtained. Label 10 a test-tube containing water only.

B takes water only and the weakest solution of camphor, and gives first one, then the other, to *A* to smell, and records the answer. Then similarly with the solution next stronger in the series, and so on but not using any solution stronger than that which is said to be smelt. (When the minimal strength of solution is thus approximately determined *A* and *B* should change rôles, to allow an interval before the final determination.) Then *B* takes water and the apparently minimal solution, and offers them to *A* five times each in indeterminate order. If *A* is right four times out of five with each fluid, test similarly with the next weakest solution.

DEMONSTRATION.

Vertical section of olfactory mucous membrane of mammal (Flemming's fluid, Ehrlich-Biondi stain).

LESSON XXXIV.

THE EYE.

A. DISSECTION.

1. Take a fresh eye of an ox or sheep, and note

The transparent **cornea**. Surrounding and continuous with this, the dirty-white **sclerotic** which forms the outer coat of the rest of the eye; the posterior two-thirds will probably be covered with fat.

The **conjunctiva**, a continuation of the mucous membrane of the eyelids. In taking the eye out of the orbit this membrane is cut through where it passes from the eyelids to the sclerotic. Dissect it forwards in any one place; it will be traceable to the junction of the sclerotic and the cornea. (Its epithelium is continuous with that of the cornea (cp. B. § 1)).

2. Clear away the ^{fat} surrounding the four straight muscles, it will be seen that their tendons form a layer under the conjunctiva of the sclerotic.

3. Cut away the conjunctiva and muscles, and remove the fat around the **optic nerve**; this pierces

the sclerotic on the nasal side, and not in the axis of the eye.

4. Cut through the cornea close to its junction with the sclerotic and remove it; the anterior chamber of the eye, containing clear, limpid **aqueous humour**, is thus laid bare. Observe the iris with its central aperture through which projects the anterior part of the lens.

5. At a little distance from the cornea cut through the sclerotic, being careful not to cut too deeply; it will separate easily from the pigmented subjacent **choroid**, except at the junction of the sclerotic with the cornea, and at the entrance of the optic nerve. In other places there is only a loose connection, largely by means of blood vessels. Remove a strip of the sclerotic, a few mm. breadth, stretching from the optic nerve to the cornea. Note its dark inner surface, or **lamina fusca**; note also in the front part of the choroid, close to the cornea (region of the ciliary muscle), the pale fibres spreading from the junction of the sclerotic and cornea, backwards over the choroid.

6. Carefully pinch up the choroid about half-way between the optic nerve and the cornea with a fine pair of forceps, and snip it through. Underneath it will be seen a thin membrane, the **retina**. The pigment layer of the retina will probably be torn away with the choroid.

7. Tear away a piece of the retina to expose the clear **vitreous humour** which occupies the posterior cavity of the eye.

8. Extend the gap laterally and tilt the eye; through the vitreous humour will be seen the ciliary part of the retina with the choroid coat becoming folded longitudinally as it approaches the lens, and so forming the **ciliary processes**. If they are not distinctly seen, cut away more of the coats of the eye.

The nervous elements of the retina cease at the level of the commencement of the ciliary process. Their termination is marked by an uneven line, the **ora serrata**.

9. Holding up the choroid and retina, cut them through as far forward as the ora serrata; it will be seen that the vitreous humour separates readily from the retina as far as that line, but in the region beyond its thin outer coat, the **hyaloid membrane**, becomes attached to the ciliary processes. If an attempt be made to separate them here with the handle of a scalpel, it will be found that the pars ciliaris retinæ (or the non-nervous continuation of the inner coat of the retina) together with some of the pigment layer of the retina (the outer coat) will come away with the vitreous humour.

10. Turn the eye with the cornea uppermost, and cut away the free edge of the iris; make two incisions at right angles to one another on the surface of the lens, it will be seen that the lens is covered by a membrane; this is the anterior part of the **lens capsule**. Carefully remove the lens, and trace out the limits of the lens capsule, noting that it forms a complete investment for the lens.

11. Gently separate with the handle of a scalpel the lens capsule from the front part of the ciliary processes, and observe that a membrane, the **suspensory ligament** or **zone of Zinn**, passes from the edge of the capsule to the ciliary processes, of which it forms the innermost layer, dipping down into their depressions.

12. Looking into the eye from the front, observe
The entrance of the optic nerve.

The blood vessels radiating out from the entrance of the optic nerve.

The iridescence, mainly below the entrance of the optic nerve; it is caused by the irregular reflection of light from the wavy connective-tissue fibres of the choroid. In this region (the tapetum) the hexagonal cells of the retina have no pigment.

13. Separate the rest of the retina from the choroid, and observe that

The pigment-layer generally adheres rather to the choroid than to the retina.

Apart from the pigment-layer the retina appears like an expansion of the optic nerve.

The retina is firmly attached to the choroid at the ora serrata.

B. HISTOLOGY.

1. Section of the anterior part of the eye of cat or dog¹. Observe

¹ The eye is placed in Flemming's fluid for half-an-hour to an hour, the posterior half is then cut away: the anterior half placed again in Flemming's fluid. When hardened a cut is made in the

The irregular connective-tissue of the sclerotic passing into the lamellæ of the cornea. The fan-like bundles of fibres spreading from the junction of the cornea and sclerotic, to the iris (ligamentum pectinatum), and to the choroid. Amongst the latter bundles are some bands of unstriped muscle, the **ciliary muscle**.

The **ciliary processes**, forming a much folded membrane. Note the two layers of cells, columnar to cubical cells and pigment cells, continuous respectively with the inner coat and with the pigment cells of the retina.

The hyaline **suspensory ligament** attached to and projecting from the ciliary processes, and running to the capsule of the lens.

The passage of the layers i to ix of the retina (§ 7) to columnar epithelium at the ora serrata.

The **iris**, consisting of, (a) a layer of pigment cells (uvea) continuous with the pigment of the ciliary processes, and so of the retina; (b) a thin fibrous-looking layer, forming the **dilatator pupillæ**; (c) a thick layer of connective tissue with pigment cells, and with numerous blood vessels (note the transversely cut vessels in the peripheral part of the iris) and nerve bundles. (d) a wedge-shaped bundle of transversely cut muscle fibres near the pupillary margin, the **sphincter pupillæ**. (e) a rather indistinct anterior limiting membrane.

The **cornea**, consisting of, (a) the anterior episuspensory ligament and the lens removed; the rest is cut in radial pieces, these are stained in bulk, cut in paraffin, the sections approximately parallel with the ciliary processes alone being mounted.

thelium continuous with that of the conjunctiva; note, the external layer of flattened but nucleated cells, the middle layer of two or three polyhedral cells, and the inner layer of a single row of columnar cells. (b) the connective tissue arranged in laminæ, with flattened cells between them, it forms an inconspicuous anterior basement membrane. (c) the **posterior elastic membrane** (membrane of Descemet), thick and hyaline, with very sharp outlines. (d) the posterior epithelium of flattened cells.

2. **Lens.** Take an ox's or sheep's lens which has been in potassium bichromate .2 p.c. for a week. Note the junctional lines of the lens fibres as a star; and that the fibres readily peel off in laminæ.

3. Antero-posterior section of lens¹; stain with picrocarmine, mount in glycerine. Note

The hyaline capsule.

The columnar epithelium of the anterior surface of the lens; at the sides these rapidly but gradually elongate into fibres.

At the sides, the nuclei of the fibres forming an irregular band.

4. Section through the centre of a lens at right angles to its short axis. Observe the cross sections of the fibres, and their junctional lines.

¹ The eye of a rabbit or cat, with the cornea and sclerotic cut through, is placed in Müller's fluid for three weeks or longer; then the lens is cut out with its capsule, and vertical sections made through its centre with a freezing microtome; neither the lens nor the sections should be touched with spirit.

5. Place the lens of a rabbit or rat in osmic acid .25 p.c. for about three hours ; it will swell up somewhat, and the outer coat becomes jelly-like and easily torn ; to obviate this, place it in gold chloride .25 p.c. for a minute or less ; then tear off as long a strip as possible of the outer coat, and tease out in water. Observe the long band-like fibres with serrated edges usually adhering together in layers but in some cases isolated.

In some of the fibres a nucleus will be seen ; when the fibres form a layer, the nuclei appear as an irregular band running across it.

6. Make a moist film preparation of, and fix with alcohol, the iris of a white rat with the posterior surface of the iris uppermost ; stain with hæmatoxylin. Observe the arrangement of the sphincter muscle.

7. **Retina.** Section through posterior part of the eye of a mammal (Flemming's fluid or potassium bichromate 2 p.c.; stained in bulk cut in paraffin). Observe

(i) The **inner limiting membrane** showing as a thin line.

(ii) The layer of **optic nerve fibres**, thin, and inconspicuous ; the fibres are without medulla, a small bundle of fibres may be seen here and there, dipping towards the next layer.

(iii) The **ganglionic** layer, consisting of a single layer of nerve-cells with conspicuous nuclei.

(iv) The **inner molecular** layer, appearing as very close plexus of fine fibrils.

(v) The **inner nuclear** layer, showing two to four rows of round or oval nuclei. The cell substance is inconspicuous.

(vi) The **outer molecular** layer, a thin layer much like the inner molecular layer (iv).

(vii) The **outer nuclear** layer; the nuclei are smaller and more numerous than in the inner nuclear layer (v).

(viii). The **outer limiting membrane**, a well defined line.

(ix) The layer of **rods and cones**, probably the outer and inner limbs of the rods can be distinguished. Note the cones, shorter or less numerous than the rods.

(x) The layer of **pigment cells** enveloping the free ends of the rods.

Observe also the **supporting fibres** (fibres of Müller), seen as lines stretching a greater or less distance between the inner and the outer limiting membrane.

8. Section of posterior part of eye of newt¹. Note

The supporting fibres, having an expanded membranous base at the inner limiting membrane, an elongated nucleus in the inner molecular layer, and stretching to the outer limiting membrane.

The large outer limbs of the rods, and the processes from the pigment cells running between them.

9. Examine in detail the retina of the newt (§ 8), and compare it with the mammalian retina (§ 7). Note

¹ Suspended in osmic vapour 10 minutes, placed in Flemming's fluid about 2 hours, the anterior part removed, the posterior part passed slowly through alcohols, and imbedded. Sections stained on the slide with Ehrlich-Biondi fluid.

The ganglionic layer is represented by two rows of cells, the nuclei are conspicuous, but with very little cell substance.

The outer molecular layer is a mere line.

The nuclei of the outer nuclear layer are elongated.

The rods and cones are much larger and more easily seen than in the mammal. The outer limbs of the rods will be more deeply stained with osmic acid (if this has penetrated) than the inner limbs ; the longitudinal fluting of the outer limbs will be seen, and probably the transverse breaking into discs.

The distinct pigment cells and their processes.

10. Cut out the eye from a freshly killed frog ; holding it up by the optic nerve cut off a small part $\cdot 5$ to 1 mm. of the coats of the eye around the optic nerve. Insert the point of the scissors into the hole thus made and make two radial cuts including about a third of the eye between them, cut off the anterior part, place the posterior part inner surface downwards on a slide, between two strips of thickish paper, take off the sclerotic, fixing the pigment layer at one edge and the rest of the retina at the other ; pull them apart. If this is done quickly, the retina will be seen to be a purplish-red, due to the *visual purple* in the rods. Cover with a cover-slip having on it a small drop of salt solution, and examine at once. The mosaic formed by the ends of the rods and cones of the retina should be seen ; most of the rods are purplish red, but a few green ones will be seen. The colours soon fade ; when they have faded, remove the strips of paper and tease out the piece of retina. Note the large outer limb of the rods, its longitudinal fluting, and transverse breaking into discs, the lenticular piece following this, the refractive globule in the cones ; and the pigment granules and cells.

11. Make a similar preparation of the retina of a mammal. Note the small diameter of the rods and cones.

12. Tear off a small piece of the retina from a sheep's eye preserved in Müller's fluid, probably the pigment-layer of the retina will be left adhering to the choroid. Take a small piece of this pigment-layer, and

mount it in glycerine (or take a fresh eye, and mount the cells in salt solution). Observe *en face* the single layer of hexagonal cells with large pigment granules.

DEMONSTRATIONS.

1. Effect on the iris of stimulating the sympathetic nerve.

2. Muscles of the eye dissected in the head of a sheep or dog.

3. Section through centre of the eye¹, showing the position of the lens and other parts.

4. Ciliary processes and iris of rabbit (or of a smaller mammal) with vessels injected, mounted flat with ciliary processes uppermost.

5. Radial section of anterior part of eye of man. Note the well-developed bundles of the ciliary muscle, passing from the region of the inner anterior part of the sclerotic to the choroid. At the inner and anterior part of the muscle, some transversely cut fibres may be seen (circular muscle).

6. Vertical section of blind spot; note the fibres of the optic nerve passing through the layers of the retina to reach the inner surface.

7. Vertical section through the fovea centralis of man: note the elongation of the cones and the thinning of the other layers.

¹ A fresh eye is placed in a glass receptacle, *e.g.* a glass stopper, the depth of which is half the diameter of the eye; the vessel is filled up with gum, the eye arranged so that the optic nerve is horizontal; it is then placed in a freezing mixture; when the eye is frozen a razor is passed along the top of the vessel cutting the eye in two.

LESSON XXXV.

VISION.

1. **Retinal Image.** Remove very carefully the sclerotic and choroid from a small portion of the posterior surface of the eye of an ox or a sheep¹. Place the eye in the end of a blackened tube just large enough to hold it, with the cornea outwards.

Stand 6 to 10 yards from a window, and look towards it; an inverted image of the window will be seen on the retina; move towards the window, the image will become blurred.

2. **Accommodation.** Standing some feet before a window, close one eye and hold a needle up before the other, at a distance of about six inches, so that it is at right angles to one of the horizontal bars of the window.

Look at the window-bar, the needle will appear dim and diffuse.

Look at the needle, the window-bar will appear dim and diffuse.

¹ Or better, take the eye of an albino rabbit, without cutting away the sclerotic.

The eye can accommodate itself for either the needle or the window-bar, but not for both at the same time. The accommodation for the near object is accompanied by a distinct feeling of effort.

3. Diffusion circles and accommodation. Facing a window, or a white surface, close one eye and hold a fine needle vertically before the other. At about six inches the needle will be seen distinctly. Bring it nearer the eye, the image will be dim and diffuse, and at the same time larger. The dimness and apparent increase of size are due to **diffusion**, resulting from imperfect accommodation.

Prick a small hole in a piece of card, hold it before the eye and again bring the needle close to the eye. It will be seen distinctly at a much smaller distance than before, and at the same time will appear magnified. It will be seen more distinctly because the diffusion circles are cut off.

4. Limits of accommodation. Prick two small holes in a card 1 to 1.5 mm. apart, place it touching the nose and forehead, and look through the holes, say, with the right eye.

a. Look at the needle held about a foot away; a single, distinct image will be seen. The rays passing through the two holes are united on the retina.

b. Bring the needle closer to the eye; at a certain distance it will become double; this marks the **near** limit of accommodation.

c. Fixing the needle on a sheet of paper, walk away while looking at it through the two holes; at a certain

distance it will become double, this marks the **far** limit of accommodation. This experiment succeeds best with short-sighted people.

Compare the near and far limits of accommodation as fixed by looking at a vertical needle through horizontal holes with those fixed by looking at a horizontal needle through holes placed vertically. The results will differ according to the amount of astigmatism in the eye.

5. **Accommodation and inversion of retinal image.** (Scheiner's experiment.)

Sit facing a window. Fix a needle vertically about 12 inches in front of you. Look through the holes as in § 4.

a. Look at some distant object, two blurred images of the needle will be seen; slide the nail of the middle finger along the card, and block out the right-hand hole, the blurred image of the left-hand side will disappear.

The rays passing through the two holes have not united when they fall on the retina. Thus the image on the right side of the retina is cut out, and this is referred to the left side.

b. Hold the point of a second needle a little in front of the card, and look at this; when it is distinct, the other needle will become blurred and appear as two. Block out the right-hand hole, the right-hand image will disappear. Here the rays passing through the two holes have united and crossed before they fall on the retina, so that blocking the right-hand hole cuts

out the image on the left side of the retina, and this is referred to the opposite, *i.e.* to the right side.

6. Inversion of retinal image. Hold a card with a pin hole a short distance from the eye. Move the head of a pin from below upwards, between the eye and the pin hole; the head of the pin will be seen inverted. The pin casts a shadow on the retina; the shadow on the lower part of the retina is referred to the upper part of the field of vision.

7. Helmholtz's Phakoscope. This should be used in a dark room. *A* looks with one eye through the hole opposite the needle. *B* looks through the hole at the side. A lamp or candle is placed at some little distance from the prisms and shifted about until *B* sees on the eye of *A*, when the latter looks at a distant object, two small bright patches of light on the cornea, two larger but dimmer patches on the anterior surface of the lens, and two small and very dim (not readily seen) patches on the posterior surface of the lens.

Let *A* now accommodate for the needle in front of him, making every effort not to move the eyeball. *B* will see the two patches on the anterior surface of the lens approach each other, while the other two pair remain motionless, thus showing that during accommodation for near objects the *anterior surface* of the lens becomes more convex.

Observe that in accommodating for near objects the pupil becomes smaller, and in accommodating for far objects the pupil becomes larger.

8. **Astigmatism.** *a.* Draw on a card a star composed say of eight lines passing through the centre, the angle between each two neighbouring lines being the same and the lines of equal tint and of equal thickness.

Place this at about the distance which has been determined (§ 4) as the far limit of accommodation (if this distance is more than eight or ten yards use convex spectacles).

Probably one or more of the lines will be seen much more distinctly and with less blurring than the others. Approach gradually nearer the star, and note whether the other lines become all visible at once or in succession.

Repeat this first with one and then with the other eye closed; the astigmatism may be different in the two eyes.

Then holding the star at a distance a little greater than the near limit of accommodation, fix the centre of the star with one eye, keeping the other closed, and bring the star gradually nearer; the lines will probably not all become dim at the same moment; the line last seen with near accommodation will probably be at right angles to that first seen with far accommodation.

Instead of the star a number of parallel horizontal and parallel vertical lines may be drawn.

Or, *b.* Fix a needle vertically on a board. Looking at the needle with one eye, accommodate the eye exactly for it. Then hold another needle horizontally before the first, and move it backwards and forwards until both needles are seen distinctly at the same time. This will be found to be the case when the needles are at some distance apart.

More exact results are however obtained by Scheiner's method (§§ 4, 5).

9. Irradiation. Cut out two patches of exactly the same size, of white and of black paper.

Place the white on a sheet of black and the black on a sheet of white paper.

Place them some distance off and adjust the eye so as to throw them a little out of the range of accommodation.

The white patch will appear larger than the black one.

10. Blind Spot. Make a bold mark on a sheet of white paper, place the sheet on the table, and, closing the left eye, fix the axis of vision of the other, by steadfastly looking at the mark at a distance of about 25 cm.¹ Dip a new quill-pen in black ink and place it a little to the right of the mark. Keeping the axis of vision fixed, and the head at the same distance from the table, move the pen slowly to the right. At a certain distance it will become invisible; mark this spot on the paper. Carry the pen still farther outwards. It will again come visible; mark this spot also. The two spots will indicate the outer and inner limits of the blind spot. Similarly the upper and lower limits may be traced, and with a little practice an outline of the blind spot, showing even the commencement of the retinal blood vessels as they emerge from the edge of the optic disc, may be constructed.

¹ Fix a small square (about 5 by 5 mm.) of black paper on the point of a teasing needle.

The size of the blind spot may be calculated from the formula $\frac{f}{F} = \frac{d}{D}$, where f is the distance of the eye from the paper, F the distance of the retina from the nodal point of the eye (average = 15 mm.), d the diameter of the outline on the paper, and D the outline of the blind spot.

11. Region of Distinct Vision. Draw a circular dot about 2 mm. in diameter, and round this draw eight similar dots nearly but not quite touching it. Fix the gaze on a mark on a piece of white paper about 30 cm. distant. Close below this place the figure of nine dots, and move it downwards, keeping it about 30 cm. from the eye. At a very short distance the dots can no longer be counted; then they become a blurred patch, and probably a little farther they form a single mass.

12. Purkinje's Figures. *a.* Go into a dark room with a lighted candle: looking steadfastly with one eye towards a wall¹; hold the candle to the side of that eye so that while the eye is illuminated the image of the candle is not seen, and gently move the candle up and down. In a few seconds the subdued reddish glare caused by the candle-light will be marked by branching dark lines, which will be seen to form an exact image of the retinal vessels as seen with the ophthalmoscope. The dark lines are shadows of the blood vessels; consequently the structures in which the physiological processes which give rise to the

¹ A light-coloured wall or white blind is the best. A wall, the paper of which has any very marked pattern, should be avoided.

sensation of light begin must lie behind the retinal blood vessels.

A cup-shaped space, in which the blood vessels are absent, may with care be seen; this is the yellow spot.

Or, *b*. Turn the eye inwards towards the nose so as to expose as much as possible of the thin sclerotic behind the cornea. Let an assistant with a lens concentrate the rays of a candle or lamp on the sclerotic as far behind the cornea as possible, so that the rays may pass directly through it towards the opposite side of the eye, and gently move the focus to and fro. The same image is still more distinctly seen. The smaller the focus on the sclerotic, the more distinct the image.

If the movement of the light is stopped, the image soon fades away.

In the first method the image moves in the same direction as the light when the light is moved from side to side, but in an opposite direction when moved up and down.

In the second method the movement of the image is in the same direction as that of the light, whether up and down or from side to side.

13. **The Yellow Spot.** *a*. Maxwell's Method.

Place a moderately strong, but perfectly transparent solution of chrome alum in a flat-sided glass vessel. Resting the eye for a minute or two, suddenly look through the vessel at a white cloud. A rosy spot or cloud will appear in the centre of vision and remain for some little time, but will gradually become less distinct.

The pigment of the yellow spot absorbs the blue-green rays between the lines *E* and *F* of the spectrum, these rays removed from those passing through the chrome alum, viz. red and greenish blue, leave a rose colour.

Or, *b*. Look through a sheet of blue glass at a white sheet of paper for 5 to 10 secs., the glass being 10 to 15 cm. from the eyes; then shut one eye, keeping the other open for two or three seconds, and so alternately. In the middle of the visual field a small dark patch—often rosy at first—will be seen owing to the greater absorption of the blue rays by the pigment of the yellow spot. Around the dark patch, a lighter, larger area will be seen.

14. Colour Vision in different parts of the Retina. *A* rests his chin on a block in the centre of the circle of a perimeter and looks steadily, say, with the left eye, straight before him at a mark on the centre of the crossing arms (or axis of the arm) of the perimeter¹.

B stands on *A*'s left; he has discs .75 cm. in diameter of white, red, green, yellow, and blue.

B taking a black screen in one hand covers the posterior part of the left-hand quarter circle, slips the red disc inside the circle at 65° , exposes it for about two seconds by removing the screen; *A* states what colour he has seen; it will probably be greyish.

The observation is repeated, putting the disc 5° nearer the centre each time till *A* sees a distinct red. The red is then taken back 10° to 15° and shown again for a moment to make certain that it only appears

¹ A simple form, sufficient for the purposes of this section is easily made. A hoop is taken made of thin wood, about 5 cm. broad and with a diameter of about 65 cm.; it is cut in half, and the pieces are fixed one in a horizontal, the other in a vertical plane near the top of a vertical stand about 12 cm. high, and the inner surfaces blackened. Or the experiment can be made still more simple. The observer (*A*) stands over a table on which is a strip of black cloth. He rests his forehead on a block about 30 cm. from the cloth and looks straight down on it. *B* places the coloured discs on the cloth at various distances, uncovering them for a moment.

greyish; then a disc of blue is shown, it will probably be seen as a brilliant blue.

The other discs are then shown in succession; probably green will appear greyish, whilst white and yellow will be recognised at once as white and yellow.

B then takes the white, yellow, and blue disc, and commencing at the periphery, shows them in irregular order, till the angle at which the three colours are distinctly recognised is approximately determined.

Similar observations should be made showing the discs against the lower quarter circle.

Then a yellowish-green disc and a purple disc should be taken, and shown, beginning at the periphery, they may at first appear grey, but soon the former will appear yellow, and the latter blue.

15. Positive After-Image. When waking in the morning close and shade the eyes for a minute or two, then suddenly look at the bright window for a moment or two, and then close and shade the eyes again. The image of the window exactly corresponding to the natural one, *i.e.* with the sashes dark and the panes bright, &c., will last for some little time.

To succeed, the retina should be in rest beforehand, and the exposure to the stimulus momentary or nearly so.

Or, in the evening, having closed and shaded the eyes for some time, suddenly look at a lamp and immediately close the eyes. A similar positive after-image will be seen.

This positive after-image must not be confounded with the negative after-image which comes later. It

simply shows that the sensation is of longer duration than the application of the stimulus.

16. Negative After-Image. Look fixedly for about twenty seconds

a. At a white patch (*e.g.* white wafer) on a black ground, and then look at a white surface (or preferably pass a white surface over the whole, keeping the visual axis fixed); there will be visible a corresponding dark patch on the white ground.

b. At a black patch on a white ground, and turn to a grey surface; there will be visible a white patch on a grey ground.

c. At a red patch on a black ground, and turn to a white surface, there will be visible a blue-green patch.

And so with the other colours, the colour of the negative image will be complementary to that of the actual object.

d. At a red patch on a black ground, and turn to a yellow surface; there will be visible a green patch.

e. Look fixedly at a brightly illuminated window and then close the eye. The *positive* after-image will probably not be seen; in its place there will come the *negative* after-image with the sashes as bright lines and the panes as dark spaces. This will in turn be succeeded by coloured images.

17. Contrast. *a.* Cut out two strips of the same grey paper. Place one on white paper and the other on black paper (or better still, velvet). The strip on the

black surface will appear distinctly brighter than the other. Arrange that the two strips are close to one another, look fixedly at them for 20 seconds and then close the eyes. The after-image of the strip on the black surface will appear much darker than that of the other strip. (The difference of brightness between the two is often more apparent in the after-image than in the original strips.)

b. Place three candles in front of a white, otherwise un-illuminated surface; pass between them and the surface an opaque body with a sharp clean-cut edge, so that part of the surface is illuminated by the three candles, part by two, part by one, and part un-illuminated; stand two or three yards back and look fixedly at the junction lines of the variously illuminated surfaces; it will be seen that each area is lighter close to a darker surface and darker close to a lighter one than it is elsewhere.

18. Simultaneous Contrast. *a.* Cut out a thin cross of grey paper, and place it in the middle of a sheet of bright green paper. Cover the whole with a sheet of thin tissue-paper. The grey patch will appear pink. The exact tint of the patch will depend on the tint of the green, of which it will be the complementary colour.

Surround the grey cross with a broad, dark black rim. The effect of contrast will be lost; the grey patch will appear grey.

On a red ground the grey cross will appear green, and with the other colours similar complementary effects will be produced; but the results are most striking in the case of red and green.

The effect is greatest when the patch is grey, not white, and is always heightened by covering with tissue-paper.

b. Cut a thin strip of grey paper and place it across the junction of a red with a green paper, and cover with tissue-paper.

The grey will appear green on the red side and pink on the green side.

c. Place a sheet of white paper on a table before a window illuminated by reflection from a white cloud, not with direct sunlight. On the side of the paper opposite the window place a lighted candle, and between it and the paper place a book edge-ways, or any object which will throw a shadow on the paper. Between the paper and the window place a similar object, throwing a like shadow. The distance of the candle should be such that the two shadows are of nearly equal intensity.

The shadow from the candle, though illuminated by the white sunlight, will appear blue, the complement of the reddish yellow colour of the rest of the paper illuminated by the candle.

Through a small black tube, *e.g.* a piece of black paper rolled up, look at a point on the edge of the blue shadow so that half the field of view is blue and the other white (or yellowish). While looking let someone blow out the candle; the half of the field previously blue will now become faintly yellow, and the white (or yellowish) half will become blue.

The daylight-shadow heightens the effect on the candle shadow, but may be dispensed with.

In place of sunlight and candle, two coloured lights may be used.

In the above experiments avoid looking at the colours too fixedly and for too long a time. Otherwise the results will be modified by after-images.

19. Test for red-green blindness, by Holmgren's wools. All the wools are spread out on the table. *B* (who has been found to have normal colour-vision) gives the light green wool labelled No. 1 to *A*. *A* selects¹ six wools of the same colour as No. 1, but of different

¹ Note should be made of the wools taken up by *A* compared with the test wool and then rejected, since a slightly colour-blind person will compare wools which would not be compared by a person with normal vision.

shades (saturation). *A*, if he is red-green colour-blind, will probably select one or more of the red shades. This is tested further by *B* giving *A* the light red wool labelled No. 2, to match.

Yellow-blue Colour-blindness; a similar experiment is made, matching light yellow and light blue.

20. Colour wheel. Flicker. On a colour wheel or top put two paper discs, a white and a black, interleaved so that the combined disc is half white and half black. When the wheel is rotated slowly, the individual sectors will at first be seen; increase gradually the ratio of rotation, at first more and more sectors will be seen, and later a marked flickering (coarse flicker). With still greater rapidity this passes into a fine flicker, and then into a continuous sensation. Note the speed at which flicker ceases. When a continuous sensation is produced it will be seen that the white and black discs pass into a grey.

21. Take the wheel into a brighter light; the rapidity of rotation has to be increased to abolish flicker. Take the wheel into a darker light; flicker ceases at a lower rate. Replace the white by a grey disc, fusion occurs more readily.

Repeat with yellow and blue discs, differing greatly in brightness, flicker is marked, and only disappears with rapid rotation, though much less rapid than with the black and white. Repeat with red and blue-green discs (of approximately equal brightness), flicker is less marked and ceases sooner.

These experiments show that a greater rapidity of rotation is required to get rid of flicker, the greater the difference in brightness of the two halves of the disc.

22. Colour Equation. The yellow and blue discs and also the red and blue-green will fuse so as to give approximately grey

surfaces if the amounts of each colour are suitably adjusted by sliding one disc over the other. The greys obtained by mixing two colours in this way are always coloured to some extent owing to the pigment colours not being exactly complementary, and the coloration of the grey will become much more obvious if larger black and white discs are placed on the same wheel to form a grey background. The coloration of the grey may then be neutralised by adding a third disc to the two colours; green if the coloration is inclined to red; red if inclined to green. The relative amounts of the three coloured discs on the one hand and of the black and white discs on the other may then be adjusted till the whole surface becomes a uniform grey. The quantities of each colour may then be read off by means of a scale graduated in degrees and expressed in the form of an equation, as in the following instance.

$$156Y + 156B + 48G = 106W + 254Bk.$$

23. Take overlapping discs of red and blue; by adjusting the relative amounts of the two, and rotate, all shades of reddish-blue, blue-purple, red-purple, bluish-red can be obtained.

24. **Colour wheel. Contrast.** Rotate a disc having alternate rings, one ring consisting of green and the other of black in one half of the circle and white in the remaining half. The white and black instead of fusing into grey will appear pink. In order to avoid after-sensations, the disc should only be uncovered and observed, when it has attained its full rate of rotation.

25. Take now a disc of alternate rings of green and white, and view when it is rotating rapidly, the pink colour will be much less distinct than in the previous experiment.

26. **Visual Illusions.** *a.* Draw a horizontal line 10 cm. long. Then, keeping the line horizontal in front of you, draw a vertical line through it of what seems to you of equal length. Measure it.

b. Draw a horizontal line about 2 cm. long; under this at a distance of about 1 cm. draw another line parallel to it and of equal length. At each end of one line draw a V, making the limbs of the two V's point towards one another (thus giving the line an arrow-head at each end); at each end of the other line draw a V, making the limbs of the two V's point away from one another. The former line will look shorter than the latter.

c. Measure on a card equal squares; without putting in the outline of the squares, fill in one with fine vertical lines one to two millimetres apart and the other with similar horizontal lines the same distance apart. Place them a short distance off, they will appear not square but oblong, the side at right angles to the direction of the lines looking longer than the side parallel to the direction of the lines.

DEMONSTRATIONS.

1. Kühne's artificial eye.
2. Colour wheel (cf. §§ 20-25), and colour matches of the colour-blind.
3. Mirror contrast.
4. The ophthalmoscope, (a) indirect method, (b) direct method.

LESSON XXXVI.

THE EAR.

1. **Dissection of semicircular canals in the Skate.** *a.* Cut through, with a strong sharp scalpel or with bone forceps, the cartilaginous roof of the skull transversely between the eyes; remove the posterior part of the roof. Opposite the hinder part of the brain the cartilage will be seen to be much thicker than elsewhere; it contains the vestibule and semicircular canals: slice it away in thin oblique sections, till one of the semicircular canals is reached; when this is done, follow the canal, removing the outer cartilaginous wall with a scalpel or a strong pair of scissors. Note

The almost transparent **membranous canal** much smaller than the cartilaginous canal in which it lies.

The **ampulla** or spindle-shaped dilatation of the membranous canal close to one end.

The opening of the semicircular canal at either end into a large membranous bag, the **utricle** lying deeply in the cartilage.

The **sacculus**, a membranous bag not so large as the utricle and separated from it only by a shallow constriction.

The rudiment of a cochlea may be seen as a small projection from the anterior end of the sacculus.

b. Trace out the three 'semicircular' canals, viz. the horizontal, the anterior vertical, and the posterior vertical canal; the two latter unite at their non-ampullary ends. Observe that the planes passing through these canals are at right angles to one another. A tube may be noticed running upwards from the utriculus, close to the point of entrance of the two vertical canals, to open on the surface of the body: the recessus vestibuli of mammals is homologous with this.

c. Cut through a membranous semicircular canal and pull up with forceps the part connected with an ampulla; the canal separates from the cartilage readily, but the ampulla is more firmly attached at one spot, the **crista acustica**, where a branch of the auditory nerve enters; cut through the ampulla at either end, and with a sharp scalpel cut through the nerve close to the cartilage; cut open the ampulla on the side opposite the entrance of the nerve and note the ridge of the crista acustica running transversely a third to a half way across the tube, where the nerve enters it.

d. Note the white calcareous paste, **otoliths**, within the membranous utriculus and sacculus; lift up the membranous bag and note that beneath the otoliths, nerve fibres enter through the cartilage. Remove the membranous vestibule and canals, sop up the fluid with a sponge, and moisten the cartilaginous vestibule and canals with osmic acid; in a short time the nerve fibres entering through the cartilage become blackened and so very distinct.

Trace towards the brain, cutting through the cartilage, one of the nerve bundles, *e.g.* that entering the utricle.

2. Structure of semicircular canals.

Transverse section of the crista acustica. (Flemming's fluid ; picrocarmine ; cut in paraffin.) Observe

The marked projection (conical in section) of the crista, composed mainly of homogeneous looking connective tissue.

The numerous large medullated nerve fibres running up the projection.

The cap of thicker epithelium. In this note the superficial *columnar cells*, and two or more rows of deeper lying nuclei belonging to the *fibre cells* ; here and there the slender inner and outer process of a fibre cell will be seen.

Hair-like processes projecting from the cap.

And on either side of the crista, the thinner connective tissue wall ; and the single layer of short columnar or even flattened cells.

3. Place a fresh ampulla in osmic acid .5 p.c. for about half-an-hour or longer ; treat it in the manner given in Lesson IX. § 1, *b* ; observe the nerve fibres with blackened medulla in the wall of the tube, and the shape of the isolated cells.

4. *a.* **Dissection of the internal ear of a mammal.** In a recently killed cat¹, cut away all the soft tissues surrounding the tympanic bulla, cut off the external meatus as close to the skull as possible, look

¹ The details of arrangement differ considerably in different animals ; the more general features may however be made out in other mammals.

down it and observe the **tympanic membrane**; it is placed obliquely to the canal and faces forwards, outwards, and downwards. The attached handle of the malleus may be seen shining through it.

b. Place the head with the lower jaw uppermost, and with a strong pair of forceps break away piecemeal the projecting part of the bulla. The cavity thus exposed has a floor¹ made irregular by projections. Note in the centre a projection of somewhat yellowish bone running in the long axis of the bulla; this is the **promontory** of the **cochlea**: at a lower level, in the exterior and posterior part of it, will be seen a round depression, the **foramen rotundum**.

c. In front and outside the cochlea is a projection of thin bone which prevents the membrana tympani from being seen; it divides the tympanic cavity into two parts communicating by an aperture over the foramen rotundum (this arrangement is a characteristic of the Felidæ); break through the bone from above and very carefully remove it in pieces with forceps. The rest of the cavity of the tympanum is thus exposed. Note the funnel shape of the membrana tympani; it has the handle of the malleus attached to it; this proceeds from the outside attachment of the membrane slightly upwards to a little past the middle point of the membrane (cp. foot-notes, pp. 310, 311).

d. Running out from a bony rim behind the membrana tympani in the outer part will be seen a band of

¹ It must be particularly noticed that the words floor, roof, exterior, posterior, etc. are here used with reference to the position of the head *during the dissection*; the position of the parts when the head is placed in the normal position should also be observed.

tissue stretching to the outer, anterior edge of the bony rim surrounding the foramen rotundum; from this a thin ligament proceeds at right angles to the former band to be inserted into the head of the malleus. This is the **posterior ligament** of the **malleus**.

e. On the opposite side of the head of the malleus and in the same straight line, note the **processus gracilis** running down to the lower edge of the membrana tympani; it is attached here to the bony wall by the **anterior ligament** of the **malleus**; do not attempt to trace the ligament till the malleus is removed later on. The tissues in the straight line thus followed down form the **axis band** of the malleus, *i.e.* the axis about which the ossicula auditûs turn.

f. Proceeding from the head of the malleus nearly at right angles to the axis band, inwards and downwards is a bony process to which is attached, by a very short tendon, the **tensor tympani muscle**. Press this towards its origin with a seeker and note that it tightens the membrane. Press lightly on the end of the handle of the malleus, it has but a small excursion.

g. With a fine pair of scissors cut through the attachment of the membrana tympani except at the handle of the malleus, and with a small pair of bone forceps remove the upper part of the bony ring to which it was attached. The malleus will remain in position. The incus and stapes may be seen indistinctly deep down on the outer side. In order to make out well the relation of these small bones great care is required. Take a fine saw and proceeding from behind in a plane passing just outside the incus, saw away the

external piece of bone. Observe then—best with a lens—

The rather long slender neck, and knobbed head of the malleus.

The **incus** with two processes, one passing almost horizontally backward and by a ligament attached to the bony tympanic wall, the other proceeding upwards, and attached to the head of the **stapes**. When the bones are removed later, the saddle-shaped surface of articulation of the incus with the malleus should be observed.

The **stapes**, much more transparent than the other bones; the base fits into an oval depression, the **fenestra ovalis**. Observe the **stapedius muscle** passing backwards from the head of the stapes to the aqueductus Fallopii at the lower, outer part of the cochlea.

Take out now the ossicula auditûs, and observe further the shape of each.

h. To the inner side, rather in front of the attachment of the tensor tympani muscle, observe the opening of the **Eustachian tube**; pass a probe down it and note the pharyngeal opening.

i. With a small pair of bone forceps break away the wall of the cochlea proceeding from the foramen rotundum towards the apex of the bony cone. Observe the coils of the cochlea with the central piece of bone or **modiolus** and the projecting **lamina spiralis**. With a strong pair of forceps remove the periotic bone from the skull, and cut through the cochlea down the modiolus. Observe the nerves running up its centre.

Starting from the fenestra ovalis, the vestibule may be exposed, the openings of the semicircular canals seen and traced out in the bone, but this is not easy, and the arrangement differs but little from that in the skate.

5. **Structure of the cochlea.** Section of the cochlea¹ of a guinea-pig or other mammal cut parallel with the axis of the modiolus. Observe

The division of each turn of the cochlea into three canals by the **basilar membrane** running across from the end of the lamina spiralis, and by the **membrane of Reissner** starting from the lamina farther back; the latter will very probably have been torn through.

The following modifications of the epithelium cells of the scala media, starting from the inner side of the basilar membrane.

a. Cells passing from cubical to columnar.

b. The single **inner hair cell**, columnar with short hair-like processes, the so-called hairs arising from its free surface, its deep pointed end is more or less hidden by small cells with large nuclei.

c. The **inner** and the **outer rod of Corti**.

d. The three or four **outer hair cells**, long irregular cells, not perpendicular to the membrane, but bending inwards, with short hair-like processes projecting from the surface, and deeply seated nucleus; occasionally two nuclei are seen in a cell.

¹ Cochlea in Flemming's fluid 8 to 24 hours (expose in one place the cochlear canal), water 1 day, decalcify in 75 p.c. alcohol containing 1 p.c. nitric acid, water 1 day. Stain in bulk with acid hæmatoxylin, or alum carmine, or stain the sections on a cover slip with Renaut's eosin-hæmatoxylin.

e. The **supporting cells** between the basilar part of the outer hair cells, each sending a thin process up to the reticular membrane.

f. The rings of the reticular membrane surrounding the tops of the outer and inner hair cells.

g. Cells passing from columnar to cubical, inclining inwards, like the outer hair cells.

The **membrana tectoria** proceeding from a projection of tissue on the lamina spiralis, thence enlarging and forming a more or less distinct pad above the organ of Corti; in the preparation it will probably be considerably shrunken.

The nerves running along the lamina spiralis towards the basilar membrane, and (if the section passes through the modiolus) the group of nerve-cells—**cochlear ganglion**—at the base of the lamina spiralis.

6. Break the cochlea of a mammal in two, place the pieces in .5 p.c. osmic acid for two hours; break up the cochlea into smaller pieces, wash with water for an hour or two; place in picrocarmine for an hour; under a dissecting lens, separate portions of the membranous tube and tease them out in dilute glycerine; observe the isolated fragments and cells of the organ of Corti.

7. **Reaction time for hearing.** Arrange a drum to rotate about 10 cm. a second. Put in circuit a battery, a Morse key, to make the circuit when the button is pressed down (another key may be used but not quite so conveniently), a time-marker, and a second key, to break the circuit when it is opened. B screens off the Morse key, says 'Ready,' and one to four seconds later presses down sharply the button of the Morse key.

A stands by the other key and as soon as he hears the click of the Morse key opens his own key.

Practise this a dozen times to get accustomed to it.

Then take a tuning-fork tracing once round the drum. Arrange the marker to write on it, set the drum going and repeat the experiment. The marker will mark the make and break of the current, *i.e.* the moment of the sound and the moment at which A opens the key. The time between the two, the reaction time, is read off from the tuning-fork tracing; it should be rather less than $\frac{1}{5}$ th of a second.

8. *Reaction time for cutaneous sensation.* Arrange in circuit 2 Daniell's cells; a knock-down key (or other key breaking the circuit on opening), the primary coil of an induction machine, and a time-marker. Connect the secondary coil with electrodes. Connect with the time-marker a key, say a Morse, and a cell.

Place the electrodes on the moistened skin, or better on the tip of the tongue, and push the secondary over the primary coil, until the break shock is distinctly felt. Then proceed as in the preceding section. B silently opens the (screened off) primary key. A, as soon as he feels the induction shock, closes the time-marker key. The reaction time will be found to be greater than in the case of hearing.

DEMONSTRATIONS.

1. Section of cochlea through modiolus showing the cochlear ganglion.
2. Upper limit of range of hearing.

LESSON XXXVII.

SPINAL CORD.

1. Transverse sections of human spinal cord through the cervical swelling, the mid-thoracic region and the lumbar swelling (*e.g.* 6th C., 6 Th., 4th L.) (potassium bichromate¹, cut frozen, picrocarmine). Mount in balsam. Comparison of the sections will be easier if they are mounted on the same slide. Observe first under a low and then so far as is necessary under a high power, the following general features of the spinal cord.

The **pia mater** of connective tissue surrounding the cord; it sends into the cord numerous septa. Note the blood vessels running from the pia mater into the cord along the septa.

The **anterior** and **posterior fissures**; the pia mater folds down into the anterior fissure; it sends a septum only into the posterior fissure.

¹ The spinal cord is placed in 2 p.c. potassium bichromate for 3 to 4 weeks, washed for a day in running water, placed for a day in the dark in 30 p.c., 50 p.c., 75 p.c. alcohol, then for a week in 95 p.c. alcohol. It is kept till required for sections in 75 p.c. alcohol. It is best to remove the dura mater before cutting sections. The piece to be cut is placed for a day in gum, cut frozen, the sections stained for a day in dilute Ranvier's picrocarmine.

The entrance into the cord of the **anterior roots** of the spinal nerves; they are seen as several small inconspicuous bundles running longitudinally through the white substance.

The entrance into the cord of the **posterior roots** of the spinal nerves in a compact mass.

The **white substance**, consisting chiefly of medullated nerve-fibres, forming the outer part of the cord and divided on each side by the entrance of the nerve roots into **anterior, lateral** and **posterior columns**; since the fibres of the anterior root do not enter the cord in one bundle there is no definite line of division between the anterior and lateral columns.

The **grey matter** projecting on each side into an **anterior** and a **posterior cornu**, but with no distinct separating line between them. The posterior cornu is divided into the neck, the head, and the apex, the latter pointing to the surface along the line of entrance of the nerve roots.

The large multipolar cells of the anterior cornu.

The **central canal**; it may be plugged up with epithelial cells.

A ring of deeply stained neuroglia around the central canal.

On either side of the central canal the posterior or **grey commissure** deeply stained.

The anterior or **white commissure** consisting of fairly large decussating nerve fibres in front of the anterior grey commissure; some may be traced from the anterior cornu of one side to the anterior column of the opposite side.

A deeply stained area, the **substantia gelatinosa**,

just internal to the apex of the posterior cornu. It will be better seen in the specimen § 3.

The *reticular area* in the lateral part of the posterior cornu, a little in front of the substantia gelatinosa, consisting of bundles of fibres surrounded by a small amount of grey substance.

2. Compare the three sections and note

a. General differences in the cord and the white substance.

The **transverse area of the cord** is largest in the cervical swelling, and is larger in the lumbar region than in the thoracic region.

The **transverse area of the white substance**, *i.e.* the number of nerve fibres, is greater in the cervical than in the thoracic region, and greater in the thoracic than in the lumbar region. (The latter difference may not be easily appreciable by the eye.)

Outline. The side to side diameter is larger than the antero-posterior in the cervical swelling; this is much less the case in the lumbar swelling, and the diameters are nearly equal in the dorsal region.

The **central canal** is nearer the ventral than the dorsal surface (rather more than $\frac{1}{3}$ rd way from the ventral surface in the cervical swelling and thoracic region); it is about the middle of the section in the lumbar swelling.

Division of posterior column. In the cervical region the posterior column is divided into two parts, **Goll's column** (fasciculus gracilis, postero-median column) near the posterior fissure, and **Burdach's column** (fasciculus cuneatus, postero-lateral column) near the posterior cornu and posterior roots.

In the mid-thoracic region the division is still seen, though not always distinctly; it is not present in the lumbar region. The two columns may be made out by the eye by a difference of tint, when no proper septum is present. (Goll's column begins about the lower third of the thoracic region, and increases in passing upwards.)

In the lumbar region an inner part of small nerve fibres—often elliptical—is commonly marked off from the rest; it must not be mistaken for Goll's column.

b. Differences in the grey substance.

Relative amount of grey substance. The grey substance is much larger in amount in the cervical and lumbar regions than in the thoracic.

Shape of anterior cornu. In the cervical and lumbar regions the anterior cornu has a large lateral projection, in the thoracic region it forms a narrow band with nearly parallel sides. The median border is approximately parallel with the anterior fissure in the cervical and thoracic region, but curves away from it in the lumbar region.

The lateral cornu. In the thoracic region there is a small lateral projection of the grey substance, the lateral cornu, about midway between the anterior and posterior cornu. (It is less distinct in the lower thoracic region and varies in different sections.)

Shape of posterior cornu. The neck of the posterior cornu is narrow in the thoracic region, and the apex is some distance from the surface of the cord (especially in the lower thoracic region); in the cervical region it is broader, and is broken up by bundles of transversely cut fibres, and is nearer the surface than

in the thoracic region; in the lumbar region the neck is broad; the apex is near the surface, and the head forms a rather large, roundish mass.

Nerve-cells of anterior cornu. In the cervical and lumbar regions distinct groups of large nerve-cells are seen; in the thoracic region the nerve-cells are fewer, smaller, and less obviously in groups.

Clarke's column. In the thoracic region an oval mass of cells is seen near the neck of the posterior cornu, a little behind the level of the central canal. The cells are fairly large, but less obviously multipolar than those of the anterior cornu. Clarke's column is not seen in the cervical and lumbar swellings.

Cells of lateral cornu. These are only obvious in the thoracic region, they are medium to small cells elongated in the direction of the lateral cornu.

3. Section of human cord (lumbar) with the medulla of the fibres stained (cp. App., p. 370). Note

The great number of small medullated nerve fibres in the grey substance, except in the substantia gelatinosa of the head of the posterior cornu, and around the central canal; the crossing small medullated fibres in the grey commissure; the large medullated fibres crossing in the white commissure (from the anterior cornu of one side to the anterior column of the opposite); the fibres of the posterior root partly curving into Burdach's column, partly running in small bundles through the substantia gelatinosa; the bundles of fibres from the deep part of Burdach's column curving into the grey substance.

Sections of *cord of dog* corresponding roughly with those in man, § 1 (5th C. ; 9th Th. ; 6th L.). Note the following differences from the human cord,

In the cervical region the cord is round in outline, and the central canal is only slightly nearer the ventral than the dorsal surface. The outer boundary of Goll's column is concave. The neck of the posterior cornu is rather broad, and its apex is fairly close to the surface.

In the thoracic region, Clarke's columns touch the posterior fissure, thus giving an appearance of a very broad grey commissure behind the central canal. In the lumbar region a sharp angle—almost a right angle—is presented by the head of the posterior cornu towards the posterior column (this is not very different from some sections of the human cord).

Decussation of pyramids. Section of upper part of 1st cervical segment in dog. Note

The numerous bundles of fibres surrounded by grey substance about the level of the central canal; decussating bundles cut obliquely at the base of the anterior fissure, which may be very shallow; a wedge-shaped bundle of fibres more or less obliquely cut on the median side of each anterior column. (Fibres of the pyramid crossing to the lateral pyramidal tract of the spinal cord.)

Note also the increase in grey substance dorsally of the central canal; the caput of the posterior cornu forming a roundish mass near the surface of the cord; a thin cap of small fibres on the caput (descending fibres from Gasserian ganglion), the superficial group of large fibres ventrally of the caput (the direct cerebellar tract).

DEMONSTRATIONS.

1. Section of cord treated by Marchi's method (cp. App., p. 371) above a region of transverse section.

Note the black globules of medulla showing degeneration in Goll's column; in the posterior and external part of the lateral column (direct cerebellar tract); and (more diffusely) in the antero-lateral columns, chiefly near the surface (ascending antero-lateral tract).

2. Section similar to § 1, but below the point of injury. Note

The degeneration in the centre of the posterior part of the lateral column (the crossed pyramidal tract).

3. Three sections of spinal cord treated by Marchi's method above, below and through a segment of which the posterior roots of one side are in a state of degeneration. Note in the segment, the degeneration of the entering nerve-roots and of the outer portion of the column of Burdach, and of numerous fibres throughout the grey substance. On the opposite side of the cord no degeneration is seen.

Above the segment, the degeneration in a median portion of Goll's column on the side of the injury.

Below the segment, either no degeneration or a scattered degeneration in the median part of Burdach's column.

The diffuse degeneration in the antero-lateral columns at the surface of the anterior column (descending antero-lateral tract).

4. Section of foetal cord treated by Golgi's method (cp. p. 341). Note

The wide-spreading processes of the nerve-cells of the grey substance.

LESSON XXXVIII.

THE BRAIN.

A. DISSECTION OF THE BRAIN¹ OF A DOG OR OF A SHEEP.

1. Note the thick membrane, the **dura mater** covering but not attached to the brain, it dips down between the cerebral hemispheres in the great longitudinal fissure as the *falx cerebri*, and between the cerebrum and cerebellum as the *tentorium*.

2. Cut away the dura mater, being careful not to injure the nerve-roots, and observe the **pia mater**, a thin vascular membrane dipping down into the fissures of the cerebrum and cerebellum. Note that

The cerebrum overlaps the anterior part of the

¹ The brain should be removed from the skull as carefully as possible, especial pains being taken to cut the internal carotid arteries and the cranial nerves close to the skull. It is hardened by placing it in 2 p.c. potassium bichromate for 5 or 6 weeks, it is left in a stream of water for a day or two, and kept in 70 p.c. spirit. The hardening is more rapid, if, as soon as the brain is removed from the skull, the internal carotid arteries are tied, and an injection, first of salt solution and then of the hardening fluid, made into the basilar artery. If the brain is placed in alcohol instead of potassium bichromate, it is best to place it in 50 to 70 p.c. alcohol for a day or two before placing it in 95 p.c.

cerebellum (the overlapping is less in the sheep than in the dog).

The cerebellum covers the dorsal part of the **medulla oblongata**.

3. Tearing away as much of the pia mater as may be necessary, turn forward the cerebellum; the posterior dorsal surface of the medulla oblongata will be seen.
Note

The **choroid plexuses** of the fourth ventricle, appearing on either side as a very vascular projection of the pia mater (they lie immediately above the thin epithelial roof of the ventricle); tear them away to expose the posterior half of the **fourth ventricle**.

The diverging posterior columns of the spinal cord; the column of Goll (cp. Lesson XXXVII. § 2, *a*) is continued on as the **fasciculus gracilis** which forms the lateral wall of the posterior part of the fourth ventricle; laterally of this is seen the **fasciculus cuneatus** continuous with Burdach's column of the cord.

The oblique fibres running from the lateral and anterior columns over the fasciculus cuneatus and apparently blending with it; the depression between the fasciculus gracilis and the fasciculus cuneatus at the same time disappearing, so that soon after the posterior columns have diverged, a single rounded eminence, the **restiform body**, is seen.

Anteriorly the restiform bodies run into the cerebellum, constituting the **inferior peduncles** of the **cerebellum**.

4. Turn back the cerebellum, tearing away the pia mater which dips down in front of it. Observe

The **corpora quadrigemina**, consisting of two round and rather large anterior bodies, and two smaller posterior bodies. The anterior corpora quadrigemina are partially covered by the cortex of the cerebrum.

The **superior peduncles** of the **cerebellum**, one on each side, proceeding from the cerebellum, as a roundish cord and disappearing underneath the posterior corpus quadrigeminum of the same side.

The **valve of Vieussens**, a thin layer of nervous substance stretching between the superior peduncles, and covering in the anterior part of the fourth ventricle. In the anterior part of the valve the roots of the fourth nerve may be seen rising from the middle line, and curving round to reach the base of the brain.

Tear away the valve of Vieussens and observe the anterior triangular part of the **fourth ventricle**, its lateral boundaries are the superior peduncles of the cerebellum.

5. Note on the under (ventral) surface of the medulla oblongata, without tearing away the pia mater,

The **pyramids**, two rounded cords, one on either side of the median line.

The **pons Varolii**. It consists of transverse fibres forming a broad band which runs over (ventrally of) the pyramids, and it has a median shallow depression. At its lower edge are transverse fibres forming a narrow band, the **trapezium**, which dips down underneath (dorsally of) the pyramids. (In man the fibres of the trapezium are covered by the fibres of the pons.) Note on each side the continuation of the pons fibres into the **middle peduncle** of the **cerebellum**.

The **lower olives** (inferior olivary bodies), two slight oval elevations, one on each side, laterally of the pyramid and just below the trapezium. These will be seen more distinctly when the pia mater has been torn away (cp. § 10).

The **crura cerebri** or peduncles of the cerebrum, two broad, roundish bands which appear at the anterior edge of the pons, and run forwards, diverging from one another.

The round, projecting **corpus mamillare** between the diverging crura. In the dog there is a shallow median groove dividing it in two.

The *posterior perforated space* between the crura, and posterior to the corpus mamillare.

Immediately anterior to this a small area, the tuber cinereum; from this springs the funnel-shaped infundibulum leading to the round **pituitary body**; cut across the infundibulum and observe the central space, which leads into the third ventricle. (The pituitary body may have been torn away in removing the brain from the skull.)

Note that the pituitary body consists of two bodies, a posterior oval or roundish mass fitting into the concavity of an anterior body, by which it is partially surrounded.

The **optic tracts**, two flat bundles of fibres coming obliquely forward over the front part of the crura cerebri, and meeting in the middle line to form the **optic chiasma**: from this a small piece of the optic nerve will be seen coming off on each side.

6. Observe the division of the cerebellum into a median and two lateral lobes, cut through the middle

peduncle (§ 5) on each side where it passes from the cerebellum, and remove the cerebellum; divide it by a transverse cut, and note the depth of its fissures, the white substance radiating outwards in the laminae, and covered by a thin layer of grey substance, the whole having a distinctly arborescent appearance.

In the midst of the white substance of each lateral lobe may be seen a greyer area, the *dentate nucleus*; and in the white substance of the middle lobe a small, roundish, grey spot on either side of the mid-line, the *nucleus of the roof*. These nuclei are more distinct in a brain preserved in ammonium bichromate.

7. Observe now more closely the exposed fourth ventricle. It has a roughly rhomboidal shape; its posterior triangular portion is the **calamus scriptorius**; note the opening of the central canal of the spinal cord into this; note also at the anterior end of the fourth ventricle the **aqueduct of Sylvius** or **iter** underneath the corpora quadrigemina; it runs from the fourth to the third ventricle (cp. § 19).

8. Trace the chief blood vessels running in the pia mater of the under surface of the brain, tearing away the pia mater where necessary but being careful not to tear away at the same time the nerve-roots. At the upper part of the medulla will be seen two arteries. These are the **vertebral arteries**, which having given off recurrent branches along the anterior fissure of the medulla, curve round to unite in the median line; the vessel formed by their union is the **basilar artery**, and runs forward in the median line of the pons Varolii. At the front edge of this it divides into the two **posterior cerebral arteries**, each of which running obliquely forward passes to the median side of the third nerve as it springs from the crus cerebri.

Just beyond this each posterior cerebral divides into two branches. One of these runs backward; the other proceeding

forwards, is, a little behind the optic commissure, joined by the **internal carotid artery**. Each arterial trunk so formed passes round the optic commissure, and divides into the **middle cerebral artery** which crosses the olfactory lobe, its main branch running in the fissure of Sylvius (cp. C, § 2), giving off numerous branches, and the **anterior cerebral artery** which passes forwards between the front lobes of the cerebrum, having a transverse communicating branch with its fellow of the opposite side. The anastomoses between the branches of the posterior cerebral and the internal carotid arteries and those between the two anterior cerebral arteries complete the **circle of Willis**.

9. Now carefully tear away the pia mater and observe (described for the dog, there are slight differences in the sheep)

The **third** pair of nerves, arising from the inner surfaces of the crura cerebri in front of the pons Varolii (cp. § 8).

The **fourth** pair of nerves, curving round the front edge of the pons, and arising a little behind the corpora quadrigemina (cp. § 4).

The **fifth** pair of nerves, large and conspicuous, arising from the sides of the pons.

The **sixth** pair of nerves, arising along the lateral edge of the pyramids where they hide the trapezium.

The **seventh** pair of nerves, arising from the trapezium close to its junction with the pons proper, and a little posterior to, and medially of the fifth.

The **eighth** pair of nerves laterally of and posterior to the seventh; they enter in part the lateral surface of the medulla, a part will be seen passing dorsally over the restiform bodies. Note the small bundles of fibres (striæ acusticæ) passing over the restiform bodies into the grey substance of the fourth ventricle.

The **ninth, tenth, and eleventh** pair of nerves each arising by several roots which form a line on the lateral part of the medulla; this line in its lower portion approaches the line of the posterior roots of the cervical nerves; the spinal part of the eleventh nerve will be seen as a stout nerve running down the side of the cord, giving off branches as it goes to the posterior part of the lateral area, it may be traced to about the sixth cervical nerve.

The **twelfth** pair of nerves arising by several roots between the pyramids and the lower olives (sometimes they arise on the outside of the olives).

10. Observe again the ventral surface of the brain (§ 5), removing the pia mater.

11. Separate the hemispheres and observe the **corpus callosum**, noting its curve in front (genu) and its curve behind (splenium).

12. Cut away in thin slices the dorsal surface of the hemispheres, nearly down to the level of the corpus callosum, noting the outer grey substance and the inner white substance.

Make a shallow cut along each side of the corpus callosum and pull up the cortex on the outside of the cut; a space, the body of the **lateral ventricle**, will be seen; carefully cut away the roof of this space, which will then be seen to run forwards as the anterior cornu and backwards and downwards as the descending cornu of the lateral ventricle; on one side remove the cortex so as to expose the whole length of the cornua. Observe

The **nucleus caudatus** of the corpus striatum,

seen as a large rounded projection into the anterior cornu and continued backwards as a tapering mass (tail of the nucleus) on the outer side of the body of the ventricle.

The **hippocampus major**, a rounded projection of the floor and medial wall of the descending cornu.

The floor of the body of the lateral ventricle consisting of a thin lamina (cp. § 15), the **fornix** continuous posteriorly with the hippocampus.

The **choroid plexus** of the lateral ventricle covering the lateral edge of the fornix and of the hippocampus. At the junction of the body of the ventricle with the anterior cornu the choroid plexus dips down underneath the fornix.

Turn laterally the choroid plexus and observe the band of white substance running from the fornix along the edge of the hippocampus, forming the *fimbria* of the hippocampus.

Carefully pass a seeker underneath the edge of the fornix and of the fimbria; note that they can be raised from the subjacent parts and that the choroid plexus dips underneath them.

13. Gently raise the corpus callosum, a thin membrane, the *septum lucidum*, will be seen stretching from its under surface to the fornix and separating the lateral ventricles of the two sides. Cut through the corpus callosum near its posterior end and turn it forwards, cutting through the septum lucidum; indications of the formation of the septum from two membranes will be seen, in its anterior portion a narrow space, the **fifth ventricle**, may be seen.

14. Cut through the corpus callosum anteriorly and remove it.

The parts of the fornix previously noticed in each lateral ventricle are now seen to be joined in the median line forming a triangular lamina. Anteriorly

this dips down some little distance behind the genu of the corpus callosum; as it dips down it divides into two round cords, the **anterior pillars** of the **fornix** (these will be better seen when the fornix is cut through (§ 15)).

The part of the fornix where these pillars are joined is called the *body*, this is small in lower mammals since the pillars soon diverge posteriorly; where they diverge they are called the *posterior pillars* of the fornix, these run on to form the fimbriae of the hippocampus (cp. § 12) and also send fibres which run over the inner surface of the hippocampus.

15. Carefully cut through the anterior portion of the fornix and turn it back, being careful not to drag with it the choroid plexus. A vascular membrane continuous with the choroid plexus will be seen underneath the fornix; this is the **velum interpositum**, it lies between the cortex and the mid-brain, *i.e.* in the so-called transverse fissure.

16. The choroid plexus of each side will be seen to curve back slightly and join with the other in the median line; the space left on either side between the choroid plexus and the anterior pillars of the fornix is part of the *foramen of Monro*. The remaining part of the foramen of Monro is the space in the median line between the anterior portions of the choroid plexuses where they join; this leads into the third ventricle (cp. § 19); the foramen of Monro is thus roughly a Y-shaped space, one limb communicating with the third ventricle and each of the other two with a lateral ventricle at the junction of the body with the anterior cornu.

The velum interpositum is seen to be continuous anteriorly with the recurved ends of the choroid plexuses about the foramen of Monro and laterally with the whole length of the choroid plexus of the lateral ventricles, in fact the choroid plexuses are only the free borders of the velum interpositum curving over the

edge of the fornix and over its posterior pillars. (It is to be remembered that since there is an epithelial membrane running from the fornix over the choroid plexuses to the edge of the nucleus caudatus, the lateral ventricle has no opening except at the foramen of Monro.)

17. In the median line cut through the fornix, with the posterior part of the corpus callosum which remains attached to it ;

Note that the cortex curls a short way underneath the corpus callosum.

18. On one side pull the fornix and hippocampus backwards ; note on the outer (lower) surface of the cortex, the **dentate** or **hippocampal** fissure ; it is shallow and is situated a short distance from the edge of the fimbria, nearly opposite the middle of the hippocampus ; it runs from the cortex underneath the corpus callosum (cp. § 17) to the extremity of the cortex of the descending cornu of the lateral ventricle ; the projection of the hippocampus seen in the descending cornu is caused by the folding of the cortex round this fissure ; note the lower surface of the posterior pillars of the fornix.

19. Turn back the velum interpositum ; in separating the velum posteriorly, note two projections downwards into the median space, these are the **choroid plexuses** of the **third ventricle**.

The **optic thalamus** will now be seen on each side, a depression runs round its lateral boundary ; between the optic thalami is a narrow space, the **third ventricle**. Note the tail of the nucleus caudatus stretching backwards for some distance laterally of the optic thalamus.

Note at the posterior part of the optic thalami in the median line the round **pineal gland** and the large **middle cerebral commissure**, running across the third ventricle between the optic thalami, it is of grey substance and hence very readily torn through.

Note the *peduncles* of the pineal gland, one on either side, running from the pineal gland over the optic thalamus near the middle line.

20. Cut away on one side the cortex so as to completely expose the optic thalami and optic tract, and trace the course of the optic tract, carefully tearing away the pia mater. The optic tract will be seen to curve dorsally and become continuous with an eminence, the **external corpus geniculatum**, at the posterior lateral part of the optic thalamus; over this may be seen fibres running to the optic thalamus, and a rather large band of fibres curling back and running into the anterior corpus quadrigemina (the brachium of the ant. corp. quad.).

21. Below and behind the external corpus geniculatum note a small eminence, the **internal corpus geniculatum**.

Both geniculate bodies are more marked in the dog than in the sheep; the position of these bodies is different in man; in man the backward projection of the optic thalamus or pulvinar has the position here occupied by the external geniculate body.

Disappearing underneath the posterior edge of the internal corpus geniculatum will be seen a band of white fibres, the brachium of the post. corp. quad.

22. Note the **anterior cerebral commissure**, a small compact bundle of fibres running transversely in front of the anterior pillars of the fornix. Cut through the anterior pillars of the fornix to expose the commissure.

23. Note the **posterior cerebral commissure** underneath the pineal gland.

24. Cut through the anterior and middle commissures and trace the cavity of the third ventricle into the infundibulum; cut through the posterior commissure and the corpora quadrigemina and trace out the aqueduct of Sylvius.

B. PARTS OF THE BRAIN SEEN IN SECTIONS¹.

1. Divide a brain in half by a longitudinal section carried carefully through the median line. Observe the relative positions of the structures visible on the cut surface, noting

The obliquely cut fibres in the decussation of the pyramids; the transversely cut fibres of the pons and of the trapezium; the valve of Vieussens; the corpora quadrigemina; the aqueduct of Sylvius; the posterior commissure, this will be continuous with transversely cut fibres forming a layer in the anterior corpus quadrigeminum; the pineal gland; the large middle commissure occupying a considerable portion of the third ventricle; the corpus callosum; the septum (septum lucidum), deep anteriorly, between the corpus callosum and the fornix; the pia mater entering the transverse fissure, forming there the velum interpositum which lies underneath the fornix and over the optic thalami; the anterior pillar of the fornix curving downwards in the direction of the corpus mammillare; the anterior commissure a little in front of the apparent termination of the anterior pillar of the fornix; the optic chiasma.

¹ A brain which has been hardened in ammonium or potassium bichromate should be taken.

2. Cut a transverse section of the medulla oblongata passing a little behind the posterior corpus quadrigeminum, and through the anterior part of the pons. Note

The fibres of the pons, seen only in the ventral part of the section, since they curve posteriorly as they rise dorsally.

The bundles of the pyramid, cut transversely, amongst the pons fibres.

Grey substance amongst the fibres of the pons, the *nucleus of the pons*.

A curved band of transversely cut fibres dorsally of the pons,—the *fillet*.

The comma-shaped mass of transversely cut fibres in the dorso-lateral part of the section,—the *superior cerebellar peduncle*.

3. Cut a transverse section of the mid-brain passing a little in front of the pons and through the anterior corpora quadrigemina. Observe

Ventrally, and a little removed from the median line on each side, the **crusta** of the cerebral peduncle, it forms a slight projecting oval mass of transversely cut fibres.

A thin grey layer, the **substantia nigra** above the crusta.

The **central grey substance** around the aqueduct of Sylvius.

The **tegmentum**, between the substantia nigra and the central grey substance, elsewhere it does not show distinct boundaries.

The internal corpus geniculatum, seen as a round lateral projection, will probably be cut through in the section.

4. Cut a brain through transversely, carrying the cut a little in front of the corpus mammillare and across the Sylvian fissure (cp. C. § 2). Note on surface exposed

The band of white substance, the **inner capsule**, laterally of the optic thalamus.

The bands of white substance, **corona radiata**, continuous with the inner capsule and spreading out into the convolutions of the cortex.

The corpus callosum, stretching between the coronæ radiatæ of the two sides.

The **nucleus caudatus** of the corpus striatum, a roundish, grey mass, dorsal and lateral to the optic thalamus, and partially surrounded by the deep white band of the corona radiata; it may be very small in this section.

The **nucleus lenticularis** of the corpus striatum; this occupies a small triangular space just outside the inner capsule, and has a number of fibres running transversely through it from the inner capsule; on its lateral margin is a thin white band, the outer capsule.

The anterior pillar of the fornix will also be seen cut across, near the ventral surface of the grey substance close to the third ventricle; note also the position of the fornix.

C. SOME CONVOLUTIONS AND FISSURES OF THE DOG'S BRAIN.

1. Observe on the ventral, anterior surface of the brain the **olfactory bulb** (this may have been cut off) continuous posteriorly with the **olfactory tract**; pull this a little outwards, it is unconnected with the brain except posteriorly where it runs into

the **olfactory lobe** ; this is continuous with a somewhat pear-shaped bulging, the lateral part of the uncinatate lobe.

2. On the side of the brain, note the deep **Sylvian fissure** running upwards and backwards from about the apex of the part of the uncinatate lobe here seen ; around this curves the **first** or **Sylvian convolution**.

3. Between the Sylvian convolution and the great longitudinal fissure are three convolutions, the 2nd, 3rd, and 4th. The four convolutions join anteriorly and posteriorly.

4. In the anterior part of the dorsal surface of the 4th convolution, note the deep **crucial fissure** running transversely and a little forwards, follow it on the median surface of the brain.

5. The bend of the 4th convolution around the crucial fissure is the **sigmoid gyrus**, the part in front of it being the anterior limb, the part behind being the posterior limb.

6. Cut away the 1st convolution and note the surface of the cortex, **island of Reil**, lying at the bottom of Sylvian fissure.

D. HISTOLOGY.

1. Section of cerebral hemisphere of rabbit¹ (ammonium bichromate 2 p.c.), stained with picrocarmine, mount in balsam. Observe

¹ The fresh brain should be placed in ammonium or potassium bichromate 2 p.c., the fluid changed on the following day (when the brain may be cut transversely in four or five pieces) and again in a week, then left about three weeks. It should then be cut up and the pieces washed in running water for a day, then placed in the dark for two days in 30 p.c., 50 p.c., and 75 p.c. alcohol, for a week in 95 p.c. It is then placed in 70 p.c. alcohol, and kept till required for sections. Pieces are cut frozen and stained for 2 to 3 days in Ranvier's picrocarmine.

Instead of a rabbit's brain the brain of a cat or dog may be taken ; a solution of the chromium salt may advantageously be injected into the basilar artery, but even then it is not easy to preserve the natural form of the cells of the 3rd and 4th layers of the cortex ; the structure

a. The inner layer of horizontal nerve fibres (medulated) forming the white substance; (between the fibres a considerable number of leucocytes will be seen;) from this bundles of fibres at fairly regular intervals run out into the grey substance, usually ceasing to be distinct in the third layer.

b. At the outer limit of *a* a layer of fusiform nerve cells lying amongst nerve fibres and forming the 5th layer of the grey substance of the cortex.

c. Outside this, a layer of small cells of various shapes, cells with three or more obvious though small processes predominating (angular cells); these form the 4th layer of the cortex.

d. Outside (*c*) are large pyramidal cells, the process from the apex of the cell tapering off from it and often being traceable upwards for a considerable distance; from the base three, four, or more processes may be seen to proceed. The district of the large pyramidal cells forms the 3rd layer of the cortex; it will be seen that it is much thicker than any of the rest, and that generally speaking its cells diminish in size from within outwards. Small angular cells may be seen in its deepest part.

e. Outside the preceding layer is a thin layer with numerous small pyramidal cells, the peripheral process being usually distinct; this is the 2nd layer.

The 1st layer of the cortex, consisting of a fine network of fibrils showing a few very small cells.

of the cortex in the cat and dog differs also at different points more than in the rabbit, the chief points of difference being the occurrence of very large cells in groups or singly in the lower part of the third layer, and the variation in number and extent of the angular cells, which may spread into the third layer or may be inconspicuous.

Blood vessels may be made out in all portions of the cortex, they are usually most conspicuous in the outer layers of the cortex running into it from the pia mater.

2. Section of a lobule of the cerebellum extending from the surface to the inner white substance and at right angles to the direction of the folds. Observe

The inner strand of medullated nerve fibres spreading into

The nuclear layer, formed mainly of small cells closely packed together; these cells have a very small amount of cell substance, so that probably their deeply stained nuclei only will be seen.

A single layer of large, somewhat globular cells (Purkinje's cells); each has a large peripheral process which will be seen to branch, and the branches to branch again and so on, eventually extending as fine branching fibrils nearly to the surface of the cortex; close to the surface the fibrils become lost to view. Since the branches, especially the larger ones, run to some extent laterally, the processes from neighbouring cells will be seen to cross one another. In a good specimen a small process may be seen to run from the deep portion of the cells towards the nuclear layer.

The outer layer of the cortex contains, besides the fibres from the cells of Purkinje, some scattered small angular cells with relatively large nuclei; from these cells one or more small branching processes may be seen to proceed, the fibres and cells being imbedded in a close fibrillar network; in this layer numerous capillaries will be seen.

3. *Golgi's chromate of silver method modified by Cajal.* Place a small piece of the spinal cord of a chick (about 12 days after hatching) or of a foetal mammal in a mixture of 100 c.c. potassium bichromate 3 p.c. and 25 c.c. osmic acid 1 p.c., and leave for three days in the dark. Rinse for an instant with distilled water, and put at once in .75 p.c. silver nitrate, and leave for two to three days in the dark. Rinse as before, and repeat from the beginning. Cut frozen, pass the sections rapidly through alcohols, and mount in xylol balsam (it is better not to cover). Or, instead of cutting frozen, pass the tissue rapidly through alcohols, imbed and cut (cp. p. 47).

DEMONSTRATIONS.

1. Human medulla and mid-brain. Compare with that of sheep or dog, described above. Note (cp. A, § 5) the large pyramids, the very large crura cerebri, and pons; the more conspicuous projection of the lower olives.

And (cp. A, § 9). The 6th nerves arise for the lower edge of the pons, in front of the pyramid and lower olive; the 7th arises laterally of the 6th; and a considerable distance from the 5th; the 12th arises between the pyramid and the lower olive.

2. Section of cortex of cerebral hemisphere treated by the Golgi method (cp. D. § 3). (Foetal or young mammal.) Note

The pyramidal cells with numerous branching dendrites, and single axis cylinder process running towards the white matter.

3. A similar section, to show neuroglia cells.

4. Section of cerebellum treated by Golgi method. (Foetal or young mammal.) (a) cut parallel with the laminae, (b) cut transverse to the laminae.

LESSON XXXIX.

DISSECTION OF THE LARYNX.

1. Obtain a fresh larynx of a sheep or an ox from the butcher's. It will probably be obtained with the upper part of the œsophagus attached, and surrounded by a mass of muscle and connective tissue.

Having slit up the œsophagus lengthways, turn back or cut away the sides and observe the opening into the larynx bounded in front by the epiglottis, at the sides by folds of the mucous membrane, and behind by the large converging yellow crests of the arytenoid cartilages. On looking down into the larynx the opening between the vocal cords, or **rima glottidis**, may be seen at some depth below. Observe that the mucous membrane of the œsophagus is continuous with that of the larynx. Bend down the epiglottis and note that the passage to the larynx is then quite closed.

2. From the posterior surface carefully remove the œsophagus with the pharyngeal muscles. On each side take up the cut sternal end of the sternothyroid muscle, dissect it upwards, and cut it away from the cartilage (the thyroid) to which it is attached.

Cut away also the thyro-hyoid muscle, which continues the line of the sterno-thyroid and runs from the thyroid cartilage to the hyoid bone. The hyoid bone and thyro-hyoidean membrane may be left. The outlines of the thyroid cartilage will now come into view, clear away the connective tissue until they are quite distinct. Note that

The **thyroid cartilage** consists of two lateral laminæ (alæ), which meet in front and diverge behind, and have their upper and lower posterior angles prolonged, forming the upper and lower cornua. Observe in front the rounded projection or Adam's apple.

3. Observe the **crico-thyroid muscle**, stretching from the posterior and lateral part of the lower border of the thyroid cartilage, to the front part of a neighbouring cartilage, the cricoid. Dissect off the crico-thyroid muscle, noting its attachments. The **cricoid cartilage** and the crico-thyroid membrane between the two cartilages will come into view.

4. Observe the articulations of the posterior cornua of the thyroid to the cricoid. Disarticulate one cornu, cut through the crico-thyroid membrane, and remove one-half of the thyroid, taking care not to injure any of the muscles. Trace out with the finger the outlines of the cricoid cartilage. Note that

It forms a complete ring, which in front is narrow, and is covered by the edge of the thyroid, but behind is deep and enclosed on either side by the thyroid.

5. On the posterior surface of the cricoid note on each side of a central ridge two laminæ of muscles, the

posterior crico-arytenoids; cut through their attachment to the cricoid, and reflecting them from below upwards, note that they are attached above to the external angles of two cartilages, the **arytenoid cartilages**, placed on the posterior upper edge of the cricoid cartilage.

6. Carefully clear away the tissue from the back of the arytenoid cartilages above the cricoid, and observe on the posterior surface of the former the **arytenoid muscle**; cutting it through the middle, which in the sheep is frequently tendinous, reflect it on either side; it will be seen to be attached to the back of each arytenoid cartilage; the posterior surface of the arytenoid cartilage will now be laid bare, and its articulation with the cricoid cartilage can be made out.

7. Looking at the larynx where the thyroid cartilage has been cut away, observe the **lateral crico-arytenoid muscle**. Cut away its attachment to the lateral portion of the upper margin of the cricoid, and, reflecting it, observe that it passes backwards and upwards from the cricoid to be inserted into the arytenoid cartilage just in front of the insertion of the posterior crico-arytenoid.

8. Clearing away the fat and connective tissue from the side of the larynx, observe the **thyro-arytenoid muscle** stretching across from the thyroid in front to the arytenoid behind. Cut it through in the middle and reflect both ends. Note its origin from the angle of the thyroid and its insertion into the

lateral surface of the arytenoid in front of the insertion of the lateral crico-arytenoid.

Remove the muscle altogether, and observe the lateral surface of the arytenoid cartilage.

9. Cut away on the same side the underlying mucous membrane; the interior of the larynx will now be laid open. On the opposite side the indistinct vocal cord will be seen passing as a pale rounded band of tissue from the anterior angle of the arytenoid cartilage to the angle of the thyroid. The inner or median surfaces of the arytenoid cartilages will be seen to bound a large oval space, called the respiratory space.

10. Clear away on one side any muscle or connective tissue still attached to the arytenoid cartilage, and observe more fully its shape, noting particularly the anterior projection or processus vocalis, the posterior lateral projection or processus muscularis, and the articulation with the cricoid.

11. Dissect *from the inside* the thyro-arytenoid muscle of the opposite side, and observe more carefully its attachments (§ 8).

(The larynx of the sheep differs materially from that of man, in the indistinctness of the vocal cords, in the absence of the false vocal cords and ventricles of the larynx, and in the peculiar crested conformation of the arytenoid cartilages).

DEMONSTRATION.

The use of the laryngoscope.

LESSON XL.

TISSUES OF REPRODUCTION.

1. **Ovary.** Longitudinal section of ovary of a mammal, *e.g.* cat, passing through the hilus (chromic acid and alcohol, stain in bulk, cut in paraffin).

Observe first with a low and then with a high power

The **germinal epithelium**, consisting of a single layer of short columnar cells covering the surface of the ovary except at the hilus.

The connective tissue radiating from the hilus throughout the ovary to form the **stroma**; in this many blood vessels are seen; towards the periphery the fibrous tissue largely disappears; immediately underneath the epithelium the stroma forms a denser layer.

Small **Graafian follicles** forming a zone a short distance below the germinal epithelium.

Deeper (older) Graafian follicles of various sizes scattered throughout the rest of the stroma, in each of these there is a more or less considerable, clear space.

One or more **corpora lutea** (unless the ovary has

been taken from a young animal). The corpora lutea vary greatly in size and appearance according to their age; at their maximal development they consist of narrow radiating threads of stroma, between which are a number of large polygonal cells; if the corpus luteum is younger there will be pigment (from effused blood) in its centre; and at a still younger stage the larger central part will contain blood, and the peripheral zone consist of a fine network of branched cells with polygonal cells in their meshes. (This is better seen in the ovary of the rabbit.)

2. Observe with a high power

a. The peripheral parts of the stroma consist chiefly of elongated spindle-shaped cells.

b. The small Graafian follicles. Note in these

The **ovum**, large and spherical, within it lies a comparatively large spherical nucleus, the **germinal vesicle**, in which may be seen a nucleolus, the **germinal spot**.

The **membrana granulosa**, a layer of flattened epithelium cells immediately surrounding the ovum.

The thin membrana propria enclosing the membrana granulosa.

In the smallest Graafian follicles the membrana propria will not be seen, in the larger ones the cells of the membrana granulosa become cubical or short columnar and the ovum has a membrane (cp. c).

c. The large Graafian follicles. Note in these

The stroma investment of the follicles, consisting chiefly of spindle-shaped cells, partly of fine fibrous tissue; inside this is the membrana propria.

The membrana granulosa, several cells deep, those next the membrana propria being short columnar cells, the rest flattened polyhedral cells.

The central space of the follicle.

The **cumulus proligerus** projecting into the space; it consists of a mass of cells much like those of the membrana granulosa and continuous with them, and it encloses

The ovum, like the ovum of the small Graafian follicles but with all its parts larger and its cell substance (beginning vitellus) more granular; it has further a distinct investing membrane which in some is much thickened so as to form a **zona pellucida**; from this the cell substance frequently shrinks in the process of hardening. The cells of the cumulus which lie next the zona pellucida are frequently arranged in a radiating manner.

Probably most of the stages between the smallest and the largest follicles will be seen in the specimen.

d. Large polyhedral cells in the stroma, something like those that occur in the interstitial tissue of the testis. (These are much less numerous in the ovary of the rabbit.)

In some of the sections taken near the hilus there may be seen lying in the stroma groups of tubules, lined with a short cubical or flattened epithelium, and cut at various angles. These are the tubules of the *parovarium*.

3. Take the fresh ovary of a mammal (preferably a large one); observe the bulgings due to the more or less ripe Graafian follicles. Holding the ovary on a glass slide, carefully prick the most prominent follicle and receive the contents on the slide. Examine *without a*

cover-slip, with simple lens or low objective. If the ovum is present it will at once be recognized. (The whole procedure is best performed under a dissecting microscope.) When one is obtained carefully cover with a cover-slip, inserting a thickish ring of paper in order to *avoid pressure*, and examine with a high power. Observe

The thick zona pellucida, with double contour (and radiating striation).

The granular cell substance (vitellus).

The transparent germinal vesicle, with its germinal spot. If the follicle be quite ripe these may have disappeared.

The cells of the cumulus proligerus attached all round the zona pellucida.

4. **Uterus.** Transverse section of cornu of uterus of cat (chromic acid and spirit, cut frozen); stain with hæmatoxylin and (slightly) with eosin. Observe

The thin external **peritoneal** coat.

The **muscular** coat, consisting broadly of an external longitudinal and an internal circular coat separated by connective tissue containing largish blood vessels. The size and arrangement of the coats vary in the different parts of the uterus and in different animals.

In most parts of the uterus the external muscular coat consists of two parts, an outer longitudinal coat, and an inner coat with fibres in various directions. The main circular coat, called above the internal, is taken to correspond with the muscularis mucosæ.

The **mucous membrane**, consisting of connective tissue covered with columnar epithelium, and containing long tubular glands, sometimes branched at their ends.

The surface cells, and most of the **gland cells** are **ciliated**, but possibly the cilia will not be seen, as they readily disintegrate.

5. Transverse section of *Fallopian tube*. Note the outer longitudinal and the inner circular muscular coat; the much folded sub-mucous and mucous membrane with glands, the surface ciliated epithelium.

6. **Mammary Glands.** Section of a portion of mammary gland (adult but not old, non-pregnant cat or rabbit; Flemming's fluid, cut frozen); stain with picrocarmine.

Observe the groups of lobules forming alveoli; the commonly large lumina of the alveoli, and their frequent connection with one another.

Under a high power, note the epithelium, flattened, cubical, or columnar in different alveoli; the cells commonly contain fat globules, and some have more than one nucleus. Where the contents of the alveoli have not tumbled out, note that they form a granular mass crowded with fat globules.

7. Section of mammary gland (alcohol, stained in bulk, cut in paraffin). Compare with § 6; the fat will have been dissolved out, the alveolar contents will be granular.

8. **Testis.** Transverse section of testis, and head of epididymis of cat¹ or dog (Müller's fluid or alcohol);

¹ The general features are better shown in a similar section in the pig, in which the connective tissue and the interstitial cells are relatively greater (formol, stain with Ehrlich-Biondi's fluid). The section is inconveniently large, but it holds together sufficiently to be cut frozen.

stain with picrocarmine (or better with Ehrlich-Biondi's fluid).

a. Observe under a low power

The **tunica vaginalis**, a thin membrane covering the greater part of the testis and firmly attached to the underlying tunica albuginea.

The **tunica albuginea**, the thick connective tissue coat of the testis.

The **mediastinum** (corpus Highmori), a patch of connective tissue seen near the centre of the section, bands of connective tissue radiate from it towards the tunica albuginea, but most become thin or branch on their way, so that the division of the testis into lobules is very imperfect.

The **tubuli seminiferi**, contorted tubes, surrounded by a little fibrous tissue and a considerable number of polygonal cells, the interstitial cells. (The interstitial cells are absent in some animals, as the rat; cp. § 2, *d.*)

The convoluted tubes unite near the mediastinum, forming the straight tubes.

The **rete testis**, into which the straight tubes open; note the irregular and anastomosing spaces.

The **efferent tubules** (vasa efferentia), rather large tubes arising from the rete (not present in every section).

The conspicuous tubes of the *epididymis* with large lumina.

b. Observe under a high power

The connective tissue structures and the interstitial cells mentioned under *a.*

The tubuli seminiferi, consisting of basement mem-

brane and several layers of cells. (The characters of the cells will be better seen in § 9.)

The straight tubes, lined by a single layer of cubical or flattened cells.

The rete testis, without special basement membrane, and lined by much flattened epithelium.

The efferent tubules and canal of the epididymis. Note the fibrous and unstriated muscle coats, both thin; the epithelium consisting of columnar ciliated cells, with some small cells near the basement membrane.

In the canal of the epididymis the cells are long and slender, but there is some variation in different animals; in the efferent tubules and in the coni vasculosi the cells are shorter and broader.

To see the masses of spermatozoa in the tubes, a section of the epididymis of the rat treated as the testis in § 9 should be made.

9. Section of testis of rat (mercuric chloride, stain deeply in bulk with hæmatoxylin, cut in paraffin). Note the slight amount of connective tissue and the absence of interstitial cells.

The epithelium varies in arrangement in different tubes. Observe the following forms.

a. An outer single layer of small cells, **lining cells** with deeply stained nuclei; next to this a single layer of larger cells, the nuclei being in some stage of division, most probably in the tangle stage, the **spermatogenic cells**; a layer consisting of four or five small cells, **spermatoblasts**, with less stained nuclei; lastly spermatozoa, the deeply stained heads of some lying close to and between the outermost layer of the spermatoblasts.

b. The lining cells are of two kinds, neither staining deeply ; one kind, the **supporting cells**, have a flattened base, they narrow on the inner side, and from them pass bands of faint lines to the lumen, thus dividing the remaining cells into columns. The spermatogenic cells are smaller and form an irregular double row.

c. Tubes with the heads of the spermatozoa in groups at various levels from that of the spermatogenic cells outwards.

10. Transverse section of the **vas deferens**. Note The thick longitudinal and circular muscular coat.

The mucous coat, slightly folded, without glands ; the epithelium cells are columnar and have no cilia.

11. **Spermatozoa**. Cut in half the fresh testis of a rat or mouse, and gently press the cut surface on a glass slide. Observe the spermatozoa, each consisting of

A head or body.

A long tapering tail or process.

A short intermediate part.

Note that the spermatozoa move by a whip-like movement of their tails.

12. Observe in like manner the spermatozoa of a frog or newt in the spring. The head is long and pointed, the intermediate part small and not very distinct ; from the intermediate part starts a filament which runs in a spiral around the long tail ; the filament is in reality the edge of a thin spiral membrane, but this is difficult to make out.

APPENDIX.

1. NOTES ON THE USE OF THE MICROSCOPE.

Bringing the section into focus. Great care should be taken to avoid lowering the objective upon the mounted specimen, since this may force the front lens of the object-glass out of position. If the front lens is thus decentred, the objective should be sent back to the maker to be re-set.

In focussing a specimen with a **low power**, place the specimen in the centre of the hole in the stage, lower the objective, so that it is a quarter of an inch or rather less from the specimen, and then raise the microscope-tube slowly till the specimen comes into view. When the specimen is focussed, note the distance of the lens from it.

In focussing a specimen with a **high power**, first find it with the low power, to bring in it the middle of the field of view. Lower the microscope-tube till the objective is rather more than $\frac{1}{16}$ th of an inch from the specimen; then slowly move the tube down by means of the fine adjustment, watching carefully for the appearance of the specimen.

It aids in catching sight of the specimen, if the

slide is moved a trifle to and fro, as it is being brought into focus.

When the section is very transparent, the edge of the cover-slip—supposing this is not surrounded by a projecting rim of balsam or glycerine—may first be focussed to find the level of the cover-slip, and then the slide moved about till a faint shadow of the section is seen. When the specimen is in focus, note the distance of the lens from it.

Before removing the slide from the stage, raise the microscope-tube, in order to avoid the chance of the front lens being brought into contact with mounting or cementing material at the edge of the cover-slip. Even if this is not done, a second specimen should not be placed under the high power without raising the microscope-tube, since the glass slides are not of constant thickness.

If the microscope-tube moves up and down in another tube, see that it moves easily; if it does not, clean the tube thoroughly.

The microscope should never be left with the objective on, and the ocular out, or dust will settle on the upper lens of the objective.

After using the microscope put it back in the box, or cover with a bell-jar.

Dust the mirror, if that is necessary: use the concave mirror if there is no sub-stage condenser; use the flat mirror if there is a sub-stage condenser.

The student should accustom himself to keeping both eyes open when using the microscope.

Use of diaphragm. With a low power, use a diaphragm with a large or moderately large aperture;

if there is no sub-stage condenser, a diaphragm is commonly not required for stained specimens.

With a high power, use a diaphragm with a small aperture; if this is not done, although the field may look brighter, the outlines of the cells and cell-structures and fibres will not be so well defined. But when it is desired to see chiefly or solely stained structures in the specimen no diaphragm should be used; the stained parts are then conspicuous, whilst the unstained parts are barely seen.

If the microscope has a sub-stage condenser, raise this so that its upper surface is level with the stage when using a high power; when using a low power, lower the condenser till it is the same distance below the specimen as the object-glass is above it.

Cleaning the front lens of an objective. A piece of soft chamois leather and a piece of silk should be kept in a small dust-tight box, for the purpose of cleaning the lenses.

Dust on the lens should be removed by lightly flicking it with silk.

Glycerine or any aqueous solution should be removed by streaming the surface with water from a wash-bottle, and then dabbing it lightly with chamois leather to dry it.

Canada balsam, turpentine, clove oil, should be removed by placing a drop of xylol or benzine on the surface of the lens; dabbing this with silk, and repeating the process several times.

When the definition of a section is not good, examine the slide and cover-slip, the upper and lower lenses of

the ocular, and the front surface of the objective to see that all of these are clean.

2. OBSERVATION OF FRESH TISSUES.

For cutting sections with the freezing microtome, cp. p. 41.

Some parts of the body are sufficiently firm to allow sections to be made free hand. When this is possible, it is to be preferred to the freezing method.

With structures that cannot be cut free hand, (*a*) a piece may be snapped off with scissors, *e.g.* the villi of the small intestine, the edge of the lobule of a gland; (*b*) a piece may be torn off with forceps, *e.g.* the coats of large blood vessels; (*c*) in the case of membranous structures a piece may be pinned out over a hole in a stage, *e.g.* the gastric glands and pancreas of the frog and newt (cp. p. 153).

For the normal fluids, cp. pp. 49, 50.

3. DISSOCIATING FLUIDS.

These are fluids which, whilst preserving certain parts of a tissue, dissolve or partially dissolve others, principally the cementing or ground substances, so that the former can be isolated by teasing or shaking. As a rule the piece of tissue so treated should be not more than one to two mm. square.

Osmic acid, .1 to 1 p.c., and dilute alcohol, 30 to 35 p.c., are dissociating fluids of general application.

After staying a short time in osmic acid, or a day or two in dilute alcohol, the pieces may be placed in picrocarmine for a day or two and teased in dilute glycerine (cp. pp. 71, 277).

The following agents are also used, the tissues for which they are most recommended are put in brackets; baryta water (fibrillæ of white fibrous tissue); 35 to 40 p.c. caustic potash

(unstripped muscle cells); 5 p.c. neutral ammonium chromate (mucous glands); .02 p.c. potassium bichromate (muscle and nerve-cells); .02 p.c. chromic acid (nerve-cells of spinal cord); Müller's fluid (olfactory cells); 5 p.c. chloral hydrate (serous glands).

4. TEASING.

A small piece of tissue only should be taken.

As a rule it is best to tease in the fluid in which the tissue has been preserved, but they may generally be teased in dilute glycerine, or in glycerine.

The teasing is as a rule easier if no more fluid is taken than that which clings to the piece of tissue; when the teasing is complete, a **small** drop of fluid is placed on the centre of the cover-slip, and this is lowered on the specimen.

It is important to place the slide on an appropriate ground; if the object is of a light tint, the slide should be placed on a piece of black paper; if it is of a dark tint, or stained, the slide should be placed on a piece of white paper.

Fine needles, *e.g.* No. 12, should be used.

The teasing is best done under a dissecting microscope, and the parts which are not required should be thrown away.

In teasing bundles of fibres, the fibres are best separated completely from one another and then arranged close together—parallel to one another, in a row.

Teased tissues mounted in water or in aqueous solutions can be temporarily preserved by surrounding the edges of the cover-slip with olive oil.

5. GENERAL DIRECTIONS FOR PRESERVING TISSUES.

a. The tissues should be removed from the body to the hardening agent as soon as possible after the death of the animal.

The tissues should not be allowed to soak, either in or out of the body in blood, serum, lymph or normal salt solution.

In many cases the tissues may be left in the body for a day after death, provided they are not surrounded by any excess of fluid. It is in fact easier to obtain the peripheral nerves (osmic acid) and the central nervous system (Marchi's method) without injury a day after death than in the fresh state.

If any blood or fluid is on the tissue, it should be removed by placing the tissue on blotting-paper.

The tissue should be divided with a sharp razor, into as small pieces as is consistent with obtaining all the parts required for examination. As a rule the pieces should be only 2 to 4 mm. in thickness. But very soft tissues—such as the brain—are generally placed whole in the hardening agent; they may be sliced as soon as the surface is sufficiently hardened, but such slices of the brain are apt to buckle.

The volume of the fluid should be 15 to 20 times that of the tissue. When a piece of tissue is kept days or weeks in a hardening agent, such as Müller's fluid, the fluid should be renewed in 1 day, and once or more later.

The tissues should be placed in a flat short bottle rather than in a narrow high one, unless they are suspended in fluid; they should be kept cool for the first day or two, then they may be warmed if it is desired to hasten the process of hardening.

***b.* Treatment after the primary hardening agent.** After treatment with the primary fixing or hardening agent, the excess of the agent is in nearly all cases removed from the tissue.

As a rule this is done by placing the tissue in a basin of water and allowing a slow stream of water from a tap to fall into the basin. It is left so for three hours to a day or two. This **washing in running water** should be carried out with tissues hardened in osmic acid, Flemming's fluid, chromic acid.

It may also be carried out with tissues hardened in chromium salts, mercuric chloride, formol, or Perenyi's fluid.

After washing out, the tissue is left for a day in each strength of alcohol 30 p.c., 50 p.c., 75 p.c. It may be further hardened in 95 p.c. or in absolute alcohol for a week or more. It is kept in 70 or 75 p.c. alcohol.

Since chromic acid, potassium bichromate and other chromium salts are precipitated by alcohol in the light, tissues hardened in fluids containing these reagents, when transferred to alcohol, should be *kept in the dark*, until the alcohol on renewal no longer becomes yellow.

c. Preparation of Solutions. It is generally advisable to make of any given substance a solution of the maximum strength likely to be required, and to dilute this when weaker solutions are needed. If the stronger solution contains x p.c. of the substance and it is required to make a weaker solution of y p.c., add $\frac{x}{y} - 1$ c.c. of water to each c.c. of the stronger solution.

It is rather better to use distilled water in making up the solutions, but in most cases this is not of much importance.

6. SIMPLE HARDENING AGENTS.

Alcohol. Alcohol is especially used for glands and for preserving the brain and spinal cord for staining nerve-cells by Nissl's method (cp. p. 130). Strong alcohol (95 p.c. or absolute) is generally used, but it causes great shrinking, especially of the outer parts of the tissue.

The shrinking is less if the tissue is placed in 70 to 75 p.c. alcohol for some hours to a day; transferred to 90 or 95 p.c. for a day or two, and then to absolute alcohol. When well hardened, the tissue may be kept in 75 p.c. alcohol.

Chromic acid. Make a 1 p.c. or a .2 p.c. solution. Tissues to be hardened in chromic acid .2 p.c. are left in it about a week. (Cp. p. 364.)

Formol or formaline is a 40 p.c. aqueous solution of formaldehyde. Dilute this 40 to 8 times, *i.e.* use a 1 to 5 p.c. solution

of formol. It has great penetrating power and fixes rapidly, and without external shrinking.

The reagent has not as yet been sufficiently tried for histological purposes, but it is perhaps best to leave a piece of tissue of moderate size not more than a day, to wash thoroughly and pass slowly through alcohols. Otherwise the staining is apt to be diffuse.

The vapour of formol may be used, as the vapour of osmic acid, to fix films, or small fragments of tissue.

Mercuric chloride. A saturated solution in water or in .75 p.c. salt solution is used. It is a good hardening agent for glands, and epithelium of skin and cornea. Tissues should not as a rule be left in it more than one to two days. They are then washed in running water (cp. § 5, 6) and passed through alcohols, kept for a week or more in 95 p.c. alcohol, then transferred to 75 p.c. alcohol till required. Unless the piece of tissue is small, the mercuric salt will not be wholly removed by this process; to facilitate its removal, a drop or two of iodine dissolved in potassic iodide is added to each of the alcohols, until the fluid is no longer decolorized. Then the alcohol is renewed.

The *iodine solution* is made as follows:—Dissolve 2 grams of potassic iodide in 100 c.c. of water, and add iodine to slight excess.

Since mercuric chloride is soluble in alcohol (in fact more soluble than in water), the tissue may be transferred direct to alcohol, this sometimes gives a better preservation of the tissue, but it demands repeated renewal of the alcohol.

Further hardening in alcohol is generally advisable.

If the sections show a mercuric precipitate, they should be treated with alcohol containing potassic iodide.

Osmic acid. The bottle to contain it should be washed with strong sodium hydrate or soft soap, then with sulphuric acid, and finally with distilled water. The tube containing the osmic acid should also be washed. Put the tube in the bottle, add water sufficient to make a 2 p.c. osmic acid solution, break the tube, and shake occasionally till the acid is dissolved. From the 2 p.c. solution make 1, .5 and .2 p.c. solutions.

Osmic acid does not penetrate well, so that a small piece only of the tissue should be taken.

If the tissue is to be stained, it should be left an hour or two only in osmic acid, washed in running water, passed through alcohols—a few hours to half-a-day for each—and cut without delay. Some tissues are sufficiently hardened by a short stay in osmic acid to be cut frozen without having been treated with alcohol. If the tissue is not to be stained, it may be left in osmic acid for a day. In the sections the nuclei are generally spherical and indistinct, whilst the nucleoli are obvious.

Tissues treated with osmic acid become darker in alcohol, and deteriorate. Sections of glandular tissues are as a rule best prepared by the freezing method and best mounted in glycerine.

Picric acid.

a. Make a cold saturated solution in water.

b. To 100 c.c. of (*a*) add 2 c.c. of concentrated nitric acid.

Leave the tissue in the fluid for one to two weeks. When the tissue is hardened, pass back through alcohols and wash out the picric acid in running water. Or pass at once through alcohols, beginning with 50 p.c.

Potassium bichromate. Make a 2 p.c. solution. This is, on the whole, the best reagent for the brain and spinal cord. The cord takes four to six weeks to harden and the brain six to eight weeks, unless it is cut into slices.

7. COMPOUND HARDENING AGENTS.

Chromic acid and alcohol. Mix equal parts of chromic acid 3 p.c. and alcohol 75 p.c. Various other mixtures are used. Make as required and filter. Tissues placed in the mixture should be kept in the dark. After four to six days, wash in running water.

Chromic and nitric acids (Perenyi's fluid).

40 parts, 10 p.c. nitric acid.

30 parts, 95 p.c. alcohol.

30 parts, 0.5 p.c. chromic acid.

The tissue is left in the fluid three to six days (cp. § 5, *b*).

Chromic and osmic acids (Flemming's mixture).

1 p.c. chromic acid	— 15 c.c.
2 p.c. osmic acid	— 4 c.c.
Glacial acetic acid	— .5 to 1 c.c.

This is a good hardening reagent for small pieces of tissue ; and it is especially used to preserve dividing nuclei. The penetration of the osmic acid is slow.

The tissue is placed in the fluid for a few hours to a day (cp. § 5, b.) If it is desired to see fat globules after hardening in Flemming's fluid, the tissue should be washed with water and cut frozen.

Potassium bichromate and cupric sulphate (Erlicki's fluid).

Potassium bichromate	2.5 grms.
Cupric sulphate	.5 grm.
Water	100 c.c.

This is sometimes used for the brain and cord, when a quicker hardening agent than potassium bichromate is required. After two days the fluid with the tissue may be kept at 38° to 40° C.

Potassium bichromate and sodium sulphate (Müller's fluid).

25 grams	potassium bichromate.
„	sodium sulphate.
1 litre	water.

Müller's fluid and formol. To Müller's fluid as above add 50 c.c. of formol. The formol should not be added until the fluid is required for use. This mixture may be used to harden organs or large pieces of tissue ; it fixes small pieces of tissue in about a day.

Chrome alum and acetate of copper (modified Weigert's fluid).

Chrome alum	2.5 gr.
Acetate of copper	5.0 gr.
Glacial acetic acid	5.0 gr.
Formol	5.0 c.c.
Water	100 c.c.

This is used for hardening the brain and spinal cord previously to cutting sections in which medullated fibres are to be stained (cp. p. 370). Sections may also be stained with hæmatoxylin etc. The hardening is complete in about a fortnight.

8. TREATMENT OF BONE AND TEETH.

Tissues containing lime salts may be treated with a hardening agent before decalcification; or treated with a reagent which fixes and decalcifies at the same time. As soon as decalcification is complete the tissue should be washed in running water for one to two days; as a rule it is best not to cut at once but to pass through alcohols, and keep in 75 p.c. to 95 p.c. alcohol for some days.

Fixing. Any fixing agent may be used, if the piece of bone is not too large, those which penetrate best such as alcohol are most generally applicable.

Decalcifying after fixation. The tissue is placed in

(a) 1 to 2 p.c. nitric acid.

(b) 75 p.c. alcohol containing 1 to 2 p.c. nitric acid.

The process may be quickened, if necessary, by using stronger acid up to 5 p.c.

(c) Strong nitric acid with phloroglucin. This is used where rapid decalcification is required; add 1 gram of phloroglucin to 10 c.c. of nitric acid 1·4 sp. gr., warm slowly and carefully, stirring the while; a rather violent solution of the phloroglucin will take place, forming a dark red fluid. To this add 100 c.c. water and 10 c.c. nitric acid. (Haug.) Foetal and young bones are decalcified in about half-an-hour; small pieces of hard adult bones in some hours: large pieces of hard bone and teeth take a day or more.

Simultaneous fixation and decalcification. The decalcification in this case is slow (one to several months). The fluid should be shaken gently every day, and renewed at intervals of a few days.

Picric acid in saturated aqueous solution gives excellent

results. After a fortnight the decalcification may be quickened by adding nitric acid up to 1 p.c.

Chromic acid. The tissue is placed in .2 p.c. chromic acid for about two days, then in .5 p.c. and this must be renewed several times. The final stages may be hastened by adding to the chromic acid, nitric acid up to 1 to 2 p.c.; or by using Perenyi's mixture.

9. STAINING FLUIDS.

Preservation of staining fluids from fungi. A fragment of thymol should be added to all aqueous solutions of stains.

Ehrlich's acid hæmatoxylin.

Hæmatoxylin	2 grms.
Absolute alcohol	100 c.c.
Water	100 c.c.
Glycerine	100 c.c.
Glacial acetic acid	10 c.c.
Alum	to saturation.

Delafield's hæmatoxylin.

4 c.c. of a saturated solution of hæmatoxylin in absolute alcohol.

150 c.c. of a solution of ammonia alum saturated in the cold.

Leave for a week, filter, and add

25 c.c. methyl-alcohol.

25 c.c. glycerine.

Mayer's hæmalum. Add

1 gram hæmatein, to
50 c.c. of 90 p.c. alcohol,
and dissolve by warming, pour into
1 litre of a 5 p.c. solution of alum.

Leave two or three days, and filter.

Time of maximum staining power. Delafield's hæmatoxylin becomes darker and increases in staining power for several months after it has been prepared; the acid hæmatoxylin

increases in staining power for a month or two; the hæmalum is at its maximum or nearly so, when first prepared.

Dilution of hæmatoxylin. It is best to dilute the several fluids, when they stain too quickly, with a mixture of the constituents of the fluid minus the hæmatoxylin, but when the dilute solution is only required for staining at the moment, and not for diluting the fluid in the bottles, a 1 p.c. solution of alum or distilled water may be used.

Comparison of the hæmatoxylin fluids. Delafield's hæmatoxylin gives perhaps the best preparations for the student, but it is apt to overstain and it requires frequent filtering, these disadvantages are much less marked in the case of acid-hæmatoxylin; the latter if used should be diluted so as to stain in 15 to 30 minutes. Hæmalum is in most cases best for staining in bulk.

Alum carmine.

Carmine.	1 gram
2·5 p.c. ammonia alum.	100 c.c.

Boil for 15 minutes. Let cool and filter.

Mayer's carmalum.

Carminic acid	1 grm.
Alum	10 grm.
Water	200 c.c.

Warm and filter.

Picrocarmine. Add 5 c.c. of ammonia to 2 grams carmine in a bottle capable of containing about 250 c.c. Stopper, shake and put aside till next day. Add slowly, shaking the while, 200 c.c. of a saturated solution of picric acid in distilled water. Put aside till next day. Add slowly, constantly stirring, 11 c.c. of 5 p.c. acetic acid. Put aside till next day. Filter; to the filtrate add four drops of ammonia, put back in the stoppered bottle. (Bourne.)

Comparison of carmine stains. Bourne's picrocarmine is the most generally useful for students; and perhaps carmalum for staining in bulk

Staining in bulk. The piece of tissue should be as small as possible consistent with its containing all the parts required. If stained with hæmatoxylin (best hæmalum), it should be left for two or three days in a solution diluted about three times; then left for a day in 1 p.c. alum, the fluid being changed once or twice.

If stained with carmine or picrocarmine it should be left in the strong solution for one to two days, and washed for a day in distilled water. The picric acid stain may be obtained in addition to that of the carmine by adding a little picric acid to the 95 p.c. alcohol used in dehydrating the tissue.

An eosin stain may be added to the stain with hæmatoxylin, by placing the tissue, after passing it through alcohols, in absolute alcohol moderately tinged with eosin. In this it is left for a day and then imbedded.

Acid-alcohol for nuclear stain. When it is desired to have the nuclei alone stained; the sections are placed in 70 p.c. alcohol containing 1 p.c. hydrochloric acid; when examination shows that the proper stage of decolorisation is reached, the sections are removed to 70 p.c. alcohol to wash out the acid.

Sections *overstained* with hæmatoxylin may be treated in the same way, or treated instead with 1 p.c. acetic acid.

Methylene blue, toluidin blue, thionin, saffranin, Spiller's purple.

a. An aqueous solution is made of such strength that a section when placed in two or three drops in a watch-glass can just be seen. This stains in a minute or two, the section is mounted in water; if overstained, the excess is taken out by 30 p.c. alcohol. The section may be mounted in glycerine tinged with the staining agent, but in a varying time the stain becomes diffuse.

b. The section is stained in an aqueous solution for half-an-hour to an hour, passed through alcohols and mounted in balsam; the stay in alcohol requires careful attention in order to obtain the proper degree of decolorisation.

c. A solution is made in 75 p.c. alcohol, the strength being tested as above. The sections are left in this for a day, then

decolorised to the required extent with 75 p.c. and stronger alcohols. A saturated solution of the substance in absolute alcohol plus an equal volume of water is used by some observers, but the advantage hardly seems sufficient to make up for the expense.

With these stains, cedar-wood oil or xylol should be used to clear the sections, and not clove oil or turpentine.

For the method of using methylene blue cp. p. 8 (basophil granules of leucocytes and connective tissues cells); p. 136 (nerve endings); p. 130 (basophil granules of nerve-cells). Thionin is especially used to stain the mucous cells of the intestinal canal, —and saffranin for staining dividing nuclei (cp. p. 369).

Nissl's methylene blue.

Methylene blue	3·75 grms.
Venetian soap	1·75 „
Water	1 litre.

For method of using cp. Lesson xv. p. 130.

Solution for fixing methylene blue, cp. p. 137. The molybdate of ammonia is dissolved by warming in water, a precipitate falls on adding hydrochloric acid, but it dissolves on shaking.

Eosin. The solution is made as in p. 367, § c; cp. p. 7 (oxyphil granules); p. 32 as diffuse plasma stain: p. 32 orange stain for red blood corpuscles.

Erythrosin (Held).

Erythrosin	1·0 gm.
Distilled water	150·0 c.c.
Glacial acetic acid	2 drops.

Ehrlich-Biondi stain. This is a mixture of acid magenta (Rubin S.); methyl-green 00; and orange G. It is best obtained solid from Grüber of Leipzig.

The acid-magenta stains connective tissue, the methyl-green stains nuclei, orange G stains hæmoglobin; other tissues are stained by one or more of the substances. It is perhaps best used with tissues hardened in mercuric chloride or alcohol. As a rule the sections when stained must be passed rapidly through

the alcohols if the methyl-green stain is to be preserved, xylol or cedar-wood oil should be used as the clearing agent.

Rubin S and picric acid.

95 p.c. alcohol	200 c.c.
Picric acid	2 grms.
Rubin S.	0.2 grm.

This is best used after rather deep staining with hæmatoxylin (cp. p. 33).

Gold chloride. The dilute acetic acid, the tartaric acid, and the formic acid methods have been given in the text, cp. pp. 50, 55, 58, 138.

Lemon-juice method. Filter fresh lemon-juice through flannel and place the tissue in a few c.c. of the filtrate for about five minutes. Place in gold chloride .5 to 1 p.c. for half-an-hour to an hour, wash, expose to light in acidulated water, or place in the dark in formic acid (Ranvier).

The tissue during and after treatment with gold chloride should be touched with metal as little as possible, it may be removed from one fluid to another on a small brush or glass rod. In cold weather, it is well to aid the reduction in acidulated water by placing it in a glass chamber at 20°—30° C.

Nitrate of Silver. Cp. text, pp. 54, 124, 147, 218. Good preparations depend largely upon exposure to a bright light soon after treatment with the silver. The tissue may be exposed to light, if the light is dim, in dilute alcohol instead of in water. If reduction is incomplete in a day, the tissue should be mounted in clove oil, and exposed to light.

10. SOME SPECIAL METHODS OF PREPARATION.

Staining for mitotic division of nuclei. A larval salamander 2 to 3 cm. long is hardened in Flemming's fluid; the body is divided transversely into two or three pieces and cut, the rest is cut transversely. The pieces may be cut frozen, and the sections stained, although pieces of the tissue are apt to be lost, or stained in bulk and cut in paraffin; or cut in paraffin and stained on the slide.

If larval salamanders are not obtainable, tadpoles freshly caught may be preserved in Flemming's or in Perenyi's fluid, the tail stained, and the epithelium of the two surfaces separated as far as possible with needles.

The best stains are perhaps saffranin and hæmatoxylin. In staining with saffranin the sections are left in Flemming's saffranin for a day; passed through alcohol to absolute alcohol containing .3 p.c. hydrochloric acid. A section is examined from time to time to see when the cell substance is decolorised, and the nuclear stain still distinct.

Osmic acid vapour for preserving mucous granules. A small bottle is half filled with 2 p.c. osmic acid, corked and left for a day. A hedgehog quill is stuck in another cork. Small pieces—2 to 4 mm. in diameter—of a fresh gland, which have not come in contact with blood or any fluid are placed on the quill, to which they will adhere. This cork replaces the first cork in the bottle, it is left for a day. The pieces of tissue are then placed in absolute alcohol for a day or longer; imbedded in paraffin and cut. The sections are stained for a day in methylene blue in 75 p.c. alcohol, and mounted in balsam.

If the sections are placed in alcohol about 70 p.c. or any lower percentage, the mucous granules swell up, they may then cohere, or be only pressed together into an apparently continuous mass, returning to the spherical form when placed once more in strong alcohol.

Staining medullated fibres in the central nervous system. Modified Weigert-Pal method. The tissue after hardening in the chrome-alum mixture (cp. p. 363) is washed out with water, frozen and cut (or imbedded in celloidin and cut).

The sections are placed successively in

- a. 1 p.c. osmic acid.
- b. 5 p.c. pyrogallie acid.

In each it is left for about half-an-hour and after each washed in water.

- c. .25 p.c. permanganate of potash.
- d. 1 p.c. oxalic acid.

In each it is left for one to two minutes as experience indicates, and after each washed in water.

Staining degenerating fibres. (Marchi's method.) The piece of central nervous system is placed in Müller's fluid or in 2 p.c. potassium bichromate for 10 days to three weeks, the fluid in this case not being changed; slices a few millimetres thick are then cut and placed for one to two weeks in a mixture of equal parts of Müller's fluid and 1 p.c. osmic acid. The pieces may be cut frozen, but they are apt to be brittle. If imbedded, celloidin should be used.

The method may be advantageously modified in the following way: the tissue hardened in Müller's fluid or potassium bichromate is soaked in the same fluid saturated with gum, then cut frozen, and the sections placed in the chromic-osmic mixture. Some of the sections may be stained with picrocarmine.

Staining sections still in paraffin. Sections cut in paraffin may be stained, before the paraffin is removed, by floating them on a staining agent.

Sections of tissues which have been hardened in alcohol or in mercuric chloride, stain with 1 p.c. aqueous solution of methylene blue, or with Ehrlich-Biondi fluid—and with other similar stains—in $\frac{1}{2}$ to $\frac{3}{4}$ of an hour; but they may be left for a day or longer, and decolorised to the proper extent by floating in dilute alcohol. Hæmatoxylin takes about a day to stain. Eosin stains rapidly and can be used after hæmatoxylin. Picrocarmine and alcohol solutions of methylene blue stain so slowly by this method that they are of little use.

The most convenient method of treatment for most purposes is to interpolate, after the sections are flattened, a stage of staining in the ordinary process of mounting an already stained section. The treatment then is as follows:

- a. The sections are flattened on warm water.
- b. They are taken up on a broad lifter and transferred to the staining solution, on which they float.
- c. When stained, they are placed on water and gently moved to remove excess of staining agent.

d. They are taken up on a cover-slip (or slide) and the water allowed to run from them.

e. The cover-slip is pressed gently between filter papers to remove as much water as possible.

f. The cover-slip is placed on a warm bath, at 35°—40° C. for about 10 minutes to dry.

g. The paraffin is melted, dissolved in xylol and the specimen mounted in balsam.

11. MOUNTING.

Albumin fixative for sections.

White of egg	50 c.c.
Glycerine	50 c.c.
Salicylate of soda	1 grm.

Shake up well and filter. The fluid filters very slowly.

Canada Balsam. Put some Canada balsam into a capsule, and place it in the warm chamber at about 65° C. for twenty-four hours to drive off all water. Let it cool, and dissolve it in a sufficient quantity of xylol to make a fairly fluid solution; it should be kept in a bottle with a ground-glass cap fitting over the neck of the bottle, instead of a stopper, as the stopper is apt to become fixed in the bottle; if any balsam is allowed to get on the neck of the bottle wet it with xylol and rub it off with a cloth.

Glycerine-jelly.

Glycerine	70 c.c.
Water	60 c.c.
Gelatine	10 grms.

Place the gelatine with the water in a porcelain capsule, heat the mixture in a small water bath over a Bunsen burner, until the gelatine is dissolved, stirring the while and taking care that the gelatine does not stick to the capsule. The water should not be allowed to boil, or at any rate for a short time only. To the hot solution add the glycerine and a drop or two of a strong alcoholic solution of thymol. If necessary, cool the mixture to

about 40° C., add the beaten up white of an egg and well mix. Then heat as before to about 90° C., stirring continuously. Filter through a hot-water filter.

The gelatine may be left for a day in the cold water to swell up, then dissolved by warming to about 40° C. The glycerine, warmed to about the same temperature, is added. Then proceed as above.

12. Transferring a number of sections from one fluid to another. In preparing a number of sections for class work, it is convenient to transfer them from fluid to fluid by means of a horse-hair filter. A piece of horse-hair tissue such as is used by wig makers, is sewn over a ring, provided with a handle. The fluid with the sections are poured on this; the sections remain, and float off readily when the filter is placed in fluid, with the surface downwards on which lie the sections.

12. INJECTION MASS.

a. Place 20 grms. of gelatine in cold water until it is well swollen, then pour off the water, and place the gelatine in a water bath at about 40° C. (covering it up to prevent evaporation) until it forms a fluid mass.

Rub 8 grms. of carmine into a paste with water, add about 10 grms. of strong ammonia and mix well, then add about 100 c.c. of water (or 2 p.c. chloral hydrate if the mass is to be kept), shake well and filter; if a suction-pump is not used it will probably take 10 to 20 hours to filter. Warm the filtrate to about 40° C. Pour it then slowly into the gelatine kept warm over a water bath, stirring continuously; when the fluids are well mixed, add gradually first strong acetic acid and then acetic acid diluted 10 times, stirring as before, until the smell of ammonia gives way to a faint smell of acetic acid.

b. Prepare gelatine as in (*a*). Take 100 c.c. of a 2 p.c. solution of Berlin blue warmed to 40° C. and pour it slowly into the gelatine kept warm on a water bath, stirring continuously.

The gelatine mixtures must of course be injected warm; the blood should be washed out of the organ to be injected with

warm salt solution ; during the injection warm salt solution should be poured over the organ or the whole animal should be immersed in warm salt solution. The injected tissue should be placed in alcohol.

13. CHEMICAL REAGENTS.

Neutral litmus. Add 100 grms. of litmus to 700 c.c. of water and boil. Pour off the water and boil again with 300 c.c. of water. Mix the two extracts and let the fluid stand for two days to settle. Decant, make the fluid distinctly acid with HCl and dialyse for at least eight days in running water.

Millon's reagent. Weigh out 50 grms. of purified mercury and an equal weight of pure strong nitric acid. Place the mercury in a flask in the closed chamber, add the nitric acid to it, and leave the mixture as long as any chemical action continues. If all the mercury is not dissolved warm it *gently* to complete the solution.

Add then to it twice its volume of water, and place aside for some hours ; a white crystalline precipitate will fall ; the supernatant fluid is Millon's reagent.

Fehling's fluid. *a.* Dissolve 103.92 grms. of *pure* cupric sulphate in warm water and add water to make up exactly a litre.

b. Dissolve 320 grms. of the double tartrate of sodium and potassium in warm water, add a little carbolic acid to prevent the growth of fungi, fill up with water to exactly a litre and filter.

c. Dissolve 150 grms. of caustic sodium hydrate in water, dilute to a litre with water. If the fluid is cloudy, filter through asbestos, or let stand and decant the clear fluid.

From the above, Fehling's fluid should be made at the time it is required for use. It will not keep. To make it, equal quantities of *a*, *b*, and *c* are mixed together. The cupric sulphate should be shaken up and a given quantity, say 100 c.c., accurately measured ; to this, 100 c.c. of *b* is added, and then *c* to make up

the volume to exactly 300 c.c. From 10 c.c. of this mixture the cupric salt is reduced by .05 gram of dextrose.

Solutions for Liebig's method of estimating urea.

a. Standard mercuric nitrate solution. Take 71.48 grms. of pure mercury, add 5 vols. nitric acid sp. gr. 1.425, and warm on water bath until the mercury is completely dissolved; evaporate the solution to a syrupy consistence, until the addition of a few drops of nitric acid no longer causes red nitrous fumes to come off, evaporate further until the fluid acquires a faint yellowish tinge. Stir and add about 10 vols. of water; if a precipitate is formed let stand, pour the clear fluid into a litre flask, to the residue add the minimal quantity of strong nitric acid necessary to dissolve the precipitate, add this to the fluid in the flask, wash the vessel out with water, add this to the previous fluid and fill up to exactly a litre.

One c.c. of this solution precipitates 10 mgs. of urea and leaves just enough mercuric nitrate over to be detected by sodium carbonate.

The method given above can only be used when the mercury is pure; if there is any doubt about this, the mercuric nitrate solution diluted to somewhat less than a litre should be titrated with a solution of pure urea and then further diluted to the proper amount.

b. Baryta mixture. Add two volumes of barium nitrate, saturated in the cold, to one volume of barium hydrate, also saturated in the cold.

c. A standard urea solution for titrating the mercuric nitrate solution may be made by drying urea over sulphuric acid, weighing out 4 grms. and adding water to make up 200 c.c. 10 c.c. of this just gives the end-reaction with 20 c.c. of standard mercuric nitrate solution. This however is only the case when the mercuric nitrate solution is neutralized by sodium carbonate immediately after being added to the urea solution. As this point is neglected in the method given in the text, it may be neglected in preparing the standard solution.

14. DESTRUCTION OF BRAIN OR OF BRAIN AND SPINAL CORD (PITHING) IN THE FROG.

When killing a frog for physiological or microscopical purposes it is sometimes advisable to remove as much blood as possible from the body and sometimes to lose as little blood as possible.

1. *Pithing and bleeding.* *a.* Cut off the head or the upper jaw with the skull with one stroke of strong scissors, thrust a seeker down the spinal canal; squeeze the body upward gently to get rid of the blood.

b. Have ready a nearly straight seeker. Hold the frog in a cloth, with the legs between the third and fourth fingers; and depress the head with the fore-finger. Thrust the point of a scalpel into the depression immediately behind the skull and so cut across the medulla. At once push the seeker into the brain and destroy it; and destroy similarly the spinal cord. Blood will flow copiously from the cut. Place the frog in a stream of water from a tap, and gently squeeze the body upwards, till the bleeding ceases.

2. *Pithing without bleeding.* Have ready some *small* fragments of cotton-wool, and fine pointed forceps. Proceed as in § 1, *b*, but as soon as the brain and cord are destroyed, thrust one or more of the pledgets of wool into the cut to stop the bleeding.

3. *Destruction of the brain only.* Proceed as in § 2, but destroy the brain only. To ensure the complete removal of the bulb it is best to destroy the nervous structures about 2 mm. down the vertebral canal. If observations on the reflex action of the spinal cord are to be made, the frog should be placed in a moist place for one to two days.

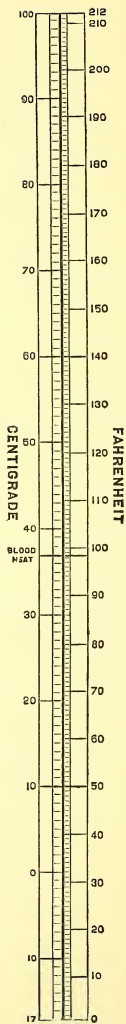
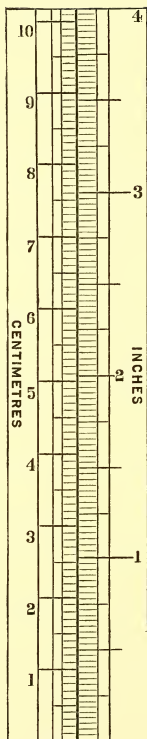
Before killing the frog in any of the preceding ways it may be anæsthetized with ether or chloroform. Take 100 c.c. of water, add 2 c.c. of ether or .2 c.c. of chloroform, shake well; in this place the frog; the limbs will gradually relax, and it will

become completely insensible. The skin may then be cut through at the junction of the skull and spinal column; the muscles behind the occipital bone cut through, the medulla exposed, before the brain or the brain and spinal cord is destroyed.

A frog, after the brain has been destroyed, may be curarized by injecting one drop of a .5 p.c. solution of curari under the skin of the back. More than is necessary should not be given if the circulation is to be observed. In the case of a newt, after the brain has been destroyed, two drops of 1 p.c. curari may be injected in the abdominal cavity with a Pravatz' syringe.

The following rough table may be useful to the student.

1 litre	= 1 cubic decimetre = $1\frac{1}{8}$ pints nearly.
1 minim	= .059 cubic centimetre.
1 pint	= 0.57 litre.
1 gallon	= 4.54 litres.
1 fluid ounce	= $\frac{1}{16}$ pint.
1 sq. inch	= 6.45 sq. centimetres.
1 cubic inch	= 16.39 cubic centimetres.
1 gram	= 15.43 grains.
1 kilogram	= 35.27 oz. Avoird. = 2.2 lbs.
1 grain	= 65 milligrams.
1 drachm	= 3.88 grams.
1 oz. Troy	= 31.1 grams.
1 oz. Avoird.	= 28.35 grams.
1 lb. Avoird.	= 0.45 kilogram.
1 centimetre	= $\frac{2}{5}$ inch.
1 metre	= 1 yard $3\frac{1}{3}$ inches.
1 inch	= 2.54 centimetres.
1 foot	= 3.05 decimetres.
1 yard	= 0.91 metre.
$\frac{1}{1000}$ inch	= $\frac{1}{40}$ millimetre about.
$\frac{1}{2000}$ inch	= 12.7 μ .
$\frac{1}{2500}$ inch	= 10.16 μ .



CHEMICAL EXAMINATION OF FLUIDS FOR CERTAIN OF THE COMMONER SUBSTANCES OF PHYSIOLOGICAL IMPORTANCE.

Notice any characteristic smell or colour.

Set a considerable portion to evaporate to dryness, completing the process on a water bath.

A. EXAMINATION FOR PROTEIDS.

1. Apply the xanthoproteic, Millon's, and the biuret tests for proteids (p. 21, *a*, *b*, *c*).

If proteids are present proceed as follows:

2. If the fluid is acid, neutralize carefully with dilute Na_2CO_3 .

If a precipitate is formed, take a few c.c. (A) and set the rest (B) to filter. To A add Na_2CO_3 to slight excess.

a. If the precipitate does not dissolve, it is not a proteid, but probably some earthy salt, *e.g.* calcium phosphate.

b. If the precipitate dissolves, wash well the precipitate from (B) on the filter with distilled water, then dissolve the precipitate in dilute Na_2CO_3 . Test the solution for proteids. If proteid reactions are

obtained, they show the presence of **acid albumin** (cp. p. 82, § 3, *a*, *b*).

3. If the fluid is alkaline, neutralize carefully with dilute acetic acid, *e.g.* 1 p.c.

If a precipitate is formed divide into A and B as in § 2. To A, add acetic acid to slight excess.

a. If a clear fluid is obtained, treat the precipitate from B as in § 2, *b*. Proteid reactions indicate the presence of **alkali albumin** (cp. p. 82).

b. If the precipitate remains on adding dilute acetic acid, it is probably due to bile acids (cp. E); if a further precipitate is formed, it is probably due to mucin (cp. p. 183) or nucleo-proteid (cp. p. 195). In such case, add B to A, make slightly acid, filter, and carefully neutralize the filtrate, if there is a precipitate test it as in (*a*) for alkali albumin.

4. To the *neutral* fluid, from which the bodies mentioned in §§ 2, 3, if present, have been removed, apply one of the tests for proteids.

If proteids are present, boil a portion, and when boiling faintly acidulate with acetic acid.

a. If no precipitate (or cloudiness) occurs, albumin and globulin are absent. If a precipitate occurs, add a few drops of strong acid (HNO_3); the solution of the precipitate indicates the presence of earthy salts (*e.g.* of phosphates); if it remains undissolved, filter, wash the precipitate on the filter, suspend in water and test for proteids by the xanthoproteic reaction. A reaction shows the presence of albumin or globulin. In this case proceed as in (*b*).

b. Saturate a portion of the neutral solution with MgSO_4 . To saturate the solution add excess of solid MgSO_4 ; stir at frequent intervals for about ten minutes. A precipitate formed at once on adding MgSO_4 may be neglected; it consists of some magnesium salt, *e.g.* a phosphate. If no precipitate is formed when the fluid is near saturation, globulin is absent, and therefore **albumin** is present.

If a precipitate occurs, a **globulin** is present. When the fluid is *completely* saturated, filter. Acidulate the filtrate with acetic acid and boil; coagulation shows the presence of **albumin**.

5. If coagulable proteids are present, make the fluid faintly acid with acetic acid, add a drop of CaCl_2 solution; boil and filter. Otherwise take the fluid used in § 4. Apply the biuret reaction, using a mere trace of CuSO_4 ; a rose tint shows the presence of **albumoses** or **peptones**. If this reaction is obtained

a. Test for **hemialbumose** by the nitric acid, and by the acetic acid and potassium ferrocyanide tests (cp. p. 194, § 6, *a*, *b*). If this is absent, **peptone** is present.

b. If hemialbumose is present, remove it by saturating the solution with $(\text{NH}_4)_2\text{SO}_4$, and filtering¹. To the filtrate apply the biuret test for **peptone**, adding twice the volume of a 40 p.c. sol. of NaHO ; the white precipitate of ammonium and sodium salts which usually occurs will not interfere with the colour test.

¹ Saturation with $(\text{NH}_4)_2\text{SO}_4$ precipitates all proteids except peptone.

B. EXAMINATION FOR FERMENTS.

1. If proteids are absent (xanthoproteic reaction not obtained), ferments are absent too.

2. If proteids are present, neutralize approximately a portion of the solution with dilute acid (HCl ·1 p.c. or less) or with dilute Na_2CO_3 and filter if a precipitate forms. Divide it into four portions.

a. Add an equal volume of HCl ·4 p.c.; divide into two portions; boil one; to each add a small piece of fibrin, already swollen in dilute HCl. Test for **pepsin** (cp. p. 191).

b. Add an equal volume of Na_2CO_3 1 p.c., and a small piece of unswollen fibrin. Test for **trypsin** (cp. p. 211).

c. Add about $\frac{1}{4}$ th volume of 1 p.c. starch. Test for **amylolytic ferment** (cp. pp. 184, 210).

d. Add an equal volume of fresh milk. Test for **rennin** (cp. p. 197).

e. Make the solution faintly alkaline with Na_2CO_3 and test for *fat-decomposing ferment* (cp. p. 211, § 7).

Place these in a water bath at about 38°C . and examine in 20 to 30 minutes.

C. EXAMINATION FOR PIGMENTS.

If the solution is coloured, examine for pigments.

1. Examine a portion with the spectroscope. The bands will show whether one or more of the following are present: oxy-hæmoglobin, carbonic oxide hæmoglobin, reduced hæmoglobin.

Distinguish **oxy-hæmoglobin** and **carbonic oxide hæmoglobin** by adding a reducing agent (cp. p. 237).

Confirm **reduced hæmoglobin** by shaking up with air (cp. p. 236).

For other derivatives of hæmoglobin cp. p. 237, §§ 10, 11.

2. Test for **bile-pigment** with Gmelin's test (cp. p. 204).

3. The urinary pigments cannot be readily distinguished in the presence of blood or bile-pigments. If these are absent, a yellow colour indicates the presence of **urochrome**. Add nitric acid and note if the tint deepens (cp. p. 247, §§ 10, 11).

D. EXAMINATION FOR CARBOHYDRATES.

If albumin or globulin is present, neutralize carefully, boil; when boiling acidulate faintly with acetic acid, filter, and test the filtrate. If unaltered starch or glycogen is present the fluid will be whitish, if glycogen alone is present the fluid will be opalescent.

1. Add iodine—a blue colour shows the presence of **starch**; a red-brown colour the presence of **dextrin** or **glycogen**; a mauve colour the presence of starch together with dextrin or glycogen (cp. pp. 184, 225, 226).

2. If a red-brown or mauve colour reaction is obtained, add to another portion twice its volume of 90 p.c. alcohol (cp. p. 226), shake, and if there is a precipitate let it stand for 5 to 10 minutes and filter.

a. To the filtrate add iodine; if a marked red-brown colour is produced, **dextrin** is present. A slight

tint may be due to incomplete precipitation of glycogen.

b. If a precipitate is obtained with alcohol, the presence of glycogen, or of glycogen plus starch is indicated. Confirm by washing the precipitate on the filter with 60 p.c. alcohol, dissolving it in a little water, and adding iodine. A slight red-brown reaction is not decisive as to the presence of glycogen, since if much dextrin is present a small amount will be precipitated by 60 p.c. alcohol.

3. If a blue colour reaction is obtained, add an equal volume of a saturated solution of $(\text{NH}_4)_2\text{SO}_4$ and filter. This will remove the starch. To a little of the filtrate add a drop of iodine. If a glycogen or dextrin reaction is obtained, add solid $(\text{NH}_4)_2\text{SO}_4$ to the remainder of the filtrate, boil to obtain a saturated solution, cool, and filter. This will remove the glycogen. Test the filtrate with iodine, a red-brown tint shows the presence of dextrin.

4. Apply Trommer's test for **reducing sugar** (dextrose, maltose, or lactose). (Cp. p. 180.) If slight reduction only is obtained, the test must be repeated after removing albumose and peptone (cp. E).

4. a. If starch, dextrin and reducing sugars are absent test directly for *cane sugar* (cp. p. 181).

b. If starch or dextrin are present and reducing sugar absent an alcoholic extract must be made (cp. E) and its aqueous solution tested for cane sugar.

c. If reducing sugars are present after starch and dextrin have been removed, indication of the presence of cane sugar may be obtained by estimating the cupric reducing power of the fluid before and after treatment with acid.

E. EXAMINATION FOR BILE-SALTS.

1. If albumose or peptone is present, treat the residue from the fluid, evaporated to dryness, with strong (*e.g.* 95 p.c.) alcohol. Filter, evaporate the filtrate to dryness in a water bath; take up the residue with water, and filter. (Proteids being insoluble in alcohol are thus removed.)

2. If coagulable proteids are present, but not albumose or peptone, get rid of coagulable proteids as in D.

3. To the fluid free from proteids, apply the furfurol or Pettenkofer's test for **bile-salts** (cp. pp. 204, 205).

F. EXAMINATION FOR UREA.

1. Test the original solution with sodium hypobromite (cp. p. 249, § *g*). If no gas is evolved, urea is absent.

2. If gas is evolved, confirm the presence of **urea**. Remove proteids as in E, § 1, up to the stage of evaporating the alcohol extract.

a. Cautiously heat a small portion of the residue from the alcoholic extract in a dry test-tube and note if NH_3 is given off, cool, and apply the biuret test (cp. p. 249, § 1, *h*).

b. Treat a part of the residue with a few drops of water, and test for crystals of urea, nitrate of urea, and oxalate of urea (cp. p. 248, §§ 1, *a*, *b*, *c*).

c. Take up the rest of the residue with water and filter, remove phosphates and sulphates if present with baryta mixture (p. 286, § 8) and test with mercuric nitrate (p. 248, § 1, e).

G. EXAMINATION FOR URIC ACID.

Remove proteids, if present, as in E. Evaporate the solution to a small bulk, and apply the murexide test (p. 255, § 1, c).

Confirm by testing for crystals of uric acid (p. 255, § 1, a).

EXAMINATION OF A POWDER OR A SOLID FOR THE COMMONER SUBSTANCES OF PHYSIOLOGICAL IMPORTANCE.

A. Place a little of the solid on a slide, cover with a cover-slip and examine microscopically. Note

1. Characteristic amorphous forms :
 - starch (concentrically or excentrically marked granules);
 - dextrin (in commercial dextrin, the form of the starch granules from which the dextrin is made is generally preserved);
 - hæmatin (dark brown scales);
 - reddish clumps indicating the presence of dried blood.
2. Characteristic crystalline forms :
 - urea (p. 248), uric acid (p. 255), leucin (p. 211), tyrosin (p. 211), cholesterin (p. 206).

Generally the crystals are more or less broken up.

3. Run iodine solution under the cover-slip.

a. Note if the colour reaction of starch, dextrin, or glycogen is given.

b. Add a drop of strong H_2SO_4 to the iodine solution. If the crystalline particles give a violet or red colour, changing to green, blue, and finally black, cholesterin is indicated (p. 206).

4. Note if many blood corpuscles are present, indicating the presence of dried blood.

If cells and fragments of tissue are present, indicating presence of dried tissue.

B. Heat a little of the solid in a dry test-tube, at first gently, then more strongly.

1. If sublimation takes place and an odour of amylamin is given off, leucin is present.

2. If an odour of phenol and nitrobenzol is given off, tyrosin is present.

3. If ammonia is given off, urea is present (p. 249).

C. Grind some of the solid in a mortar with a little cold water, add more water, stir well for a few minutes, let the residue settle, decant and filter.

1. The filtrate may contain peptone, albumose, dextrin, glycogen, sugars, bile-salts, urea, leucin.

Examine it as an ordinary physiological fluid.

2. To the residue add 1 p.c. NaHO , shake well, decant and filter.

a. The filtrate may contain nucleo-proteids, bilirubin, uric acid, tyrosin. Test for the substances individually.

If the filtrate is alkaline, due to the presence of an alkaline salt in the solid, these substances will be already more or less dissolved.

b. The residue may contain starch, cholesterin, hæmatin, hæmin, sodium urate. Boil it with water and filter hot.

a. The filtrate may contain starch. Test with iodine.

β. Concentrate the filtrate and examine for crystals of urates (p. 259).

γ. Extract the residue with boiling alcohol and filter hot.

The filtrate may contain cholesterin ; concentrate it, examine for crystals, and apply the sulphuric acid test (p. 206).

If there is a dark red or brown residue, add alcohol strongly alkaline with NaHO and boil again ; if hæmatin is present it will dissolve ; filter, examine the filtrate spectroscopically (p. 237).

D. If evidence is obtained that proteids, starch, dextrin are present in large amount, boil the solid with alcohol and filter hot. The filtrate may contain sugars, bile-salts, urea, leucin, cholesterin.

1. Concentrate the filtrate and examine for crystals of leucin (p. 211), and cholesterin (p. 206).

2. Continue the evaporation to dryness, extract the residue with water, filter, examine the aqueous extract for sugars bile-salts, urea.

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